The ecology of algal assemblages across a gradient of acid mine drainage stress on the West Coast, South Island, New Zealand

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Abstract

Physicochemical factors, algal diversity, taxonomic composition and standing crop were investigated across a broad gradient of AMD stress in streams and rivers. 52 sites were surveyed in the vicinity of Greymouth, Reefton, Westport and Blackball, on the West Coast, South Island. Seven sites in the Reefton area were sampled from April 2006 – February 2007 to establish changes over time in benthic algal communities of AMD and reference streams. Longitudinal change and ecosystem recovery were also investigated by sampling eight sites down Devils Creek, Reefton, and two of its tributaries.

AMD has negative impacts on algal diversity, generally increases the dominance of certain taxa and, where metal oxide deposition or hydraulic disturbance are not great, can lead to algal proliferations. These proliferations were chlorophyte dominated, predominantly by filamentous *Klebsormidium acidophilum*. From the general survey a total of 15 taxa were identified from the most severely impacted sites (pH <3.6), which included both acidophiles and acidotolerant algae.

Multivariate analyses strongly suggest that pH was the dominant factor controlling taxonomic occurrence of diatoms, macroalgae and the structure of the total assemblage. Other factors such as conductivity, metal oxide deposition, temperature, depth, month, geographic location and altitude were also important. Algal communities changed over time and this became more marked as AMD impact decreased. This was presumably due to AMD stressors reducing diversity, and thus the available scope for assemblage change.

Longitudinal differences in assemblage structure within Devils Creek appeared to be in response to dilution of AMD in upper reaches and to changes in natural physical features such as gradient in mid and lower reaches. After a distance of 7.2 km the physicochemical effects of AMD and suspended clay inputs were minimal. At this site and at several previous sites, the assemblage exhibited a degree of recovery towards that found at unimpacted sites.

A range of algae found in the broad scale-survey are potentially useful ‘sensitive’ indicators. These included: *Heteroleibleinia purpurascens*; *Achnanthes oblongella*; *Oedogonium* sp. and *Spirogyra* sp. In contrast: *Euglena mutabilis*; *Navicula cincta*; *K. acidophilum*; *Microspora quadrata* and *Microthamnion kuetzingianum* may be useful ‘tolerance’ indicators. These data show that AMD has a range of negative impacts on algae, and algae may be a useful tool for monitoring these impacts in West Coast streams.

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Chapter 1: AMD and algae an introduction

1.1. The scale of mining in New Zealand past, present and potential.

Mining has been occurring on the West Coast since the 1860s where there are numerous abandoned mining sites and settlements surrounding Reefton, Greymouth, Westport and Blackball. These centers were historically mining settlements themselves. Coal was first discovered by Charles Heaphy and Thomas Brunner in 1846 near Charleston. The substantial deposits on the Denniston and Stockton plateaus were discovered by Dr Julius von Haast and James Burnett in the 1860s and in 1874 the first mine in the area was established (Coal Town Museum 2006). These historic mining sites have predominantly underground shafts, many of which are still producing toxic effluent (Harding and Boothroyd 2004), the effects of which may be expected to persist for hundreds of years, until pyrite sources are exhausted (Younger 1997).

Humans are placing increasing demands upon their environment and mining is one area that is not often in the public eye despite its severe and chronic effects. Mining activities frequently damage or degrade habitats of many native species (RSNZ 2006). Where mining runoff enters streams it can have a range of negative impacts (Gray 1997; Harding and Boothroyd 2004). Minerals that have been mined in New Zealand include gravel, tin, copper, uranium, gold and coal. Underground mining is the most widespread method of mineral extraction and involves excavation of an inclined (a decline or drift) or horizontal (adit) shaft, followed by a network of perpendicular and parallel shafts (bord-and-pillar) enabling maximum extraction of the coal seam (Younger et al. 2002; Harding and Boothroyd 2004).

Opencast mining is an efficient method of mineral extraction and is commonly employed on the West Coast by the coal industry. It is a process that removes rock and soil strata above coal or other mineral deposits. This dramatically changes the landscape and creates large dumps of overburden where the excavated material may take up 50% more space than it did originally (Kelly 1988).

Alluvial mining also occurs on the West Coast and involves extracting and sifting through large amounts of old or existing river bed in order to obtain the heavy gold fines that make their way into the bed. This produces extensive areas of modified waterway and riparian zones. Extracted gravels are left in piles forming humps and hollows along stream margins. This may inhibit or prevent riparian re-vegetation, expose minerals to weathering, and alter channel morphology (Harding and Boothroyd 2004).

Each method of mineral extraction has a range of negative impacts on freshwater ecosystems (Harding and Boothroyd 2004). Underground coal mining is most often associated with acid mine drainage (AMD) runoff, while open cast and alluvial mining may change the pH very little but may dramatically increase sedimentation and turbidity of associated waterways.

Solid Energy is New Zealand's largest mining company (Solid Energy 2005). The company estimates that New Zealand's unmined coal deposits consist of approximately 15 billion tonnes of high quality coal, about 8.6 billion tonnes of which is economically mineable and may take 200 years to exhaust. The company currently extracts around 4.5 million tonnes of coal per annum. However, planned growth means that by 2012 they anticipate extracting 11.5 million tonnes per annum. Major deposits have been identified in the Waikato, West Coast and Southland (Solid Energy 2005). Brunner coal measures are the predominant coal type on the West Coast and have high quantities of associated carbonaceous mudstone which contains high concentrations of pyrite and marcasite (Edguardo 1997), while coal measures in Southland have much lower concentrations and consequently mines in that area produce effluent posing much less risk to aquatic life (pers. com. J. Harding).

AMD is known to act on long time scales and its effects and those associated with other mining activities on the West Coast are widespread. These effects will only increase as coal mining activities intensify.

1.2. Acid mine drainage chemical processes

1.2.1. Pyrite oxidation

Acid mine drainage is created where mining practices expose coal, coal tailings, overburden and rock to air and water where it is then chemically, physically and biologically weathered.

To understand how AMD is generated it is necessary to have an understanding of coal and its constituents. Coal is a term used for a continuum of organic remains that have undergone lithification during anoxic conditions (Kelly 1988). Peat is accumulated organic matter that is not broken down where bacterial action is attenuated in the anaerobic conditions of peat bogs. Due to these conditions, sulphur undergoes reduction to sulphide forming pyrite and marcasite ($Fe_2S_{(s)}$) (Kelly 1988). The sulphur content of coal is commonly within the range 0.5 to 3% (Kelly 1988). This is significant and it is these concentrations, together with metals within coal and associated rock, which often make the effluent draining mines extremely toxic.

Once pyrite ($Fe_2S_{(s)}$) is exposed the following chemical reactions take place:

$$2Fe_2S_{(s)} + 6O_{2(aq)} + 2H_2O \rightarrow 4Fe^{2+} + 2SO_4^{2-} + 4H^+ \quad \text{(a)}$$

$$Fe^{2+} + 2H_2O \leftrightarrow Fe^{3+} + O_2 + 4H^+ \quad \text{(b)}$$

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_{3(s)} + 3H^+ \quad \text{(c)}$$

$$FeS_{2(s)} + 14Fe^{3+} + 8H_2O \leftrightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad \text{(d)}$$

Steps (a, (c and (d release hydrogen ions into water decreasing the pH while step (a also increases the concentrations of soluble iron (Stumm and Morgan 1996). Step (c creates “iron hydroxide precipitate”, an orange metal oxide common in many acid mine drainage streams on the West Coast. Assuming the low pH conditions associated with many mine stressed streams, the rate determining step is step b), the oxidation of Fe^{2+} to Fe^{3+} which is usually catalyzed by chemoautotrophic bacteria (Stumm and Morgan 1996) and is thought to start in tailing waters around pH 2.5 (Salomons 1995). It is this bacterial oxidation that can help to cause such dramatic reductions in pH. Bacterial concentrations in acidic mine waters commonly range from 10^3 to 10^8 cells mL^{-1} (Johnson 2003). Espańa et al. (*In press*) believe Fe^{2+} is oxidized by the bacteria *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans.* They measured rates of oxidation between 10^{-8} and 10^{-10} mol $L^{-1}s^{-1}$ in the absence of these bacteria and observed much greater rates of oxidation (10^{-6} to 10^{-7} mol $L^{-1}s^{-1}$) in their presence. The rate was dependant upon pH, temperature and dissolved oxygen concentration, the latter of which was noted to be at its greatest where algal biomass was maximal (Espańa et al. *In press*; Garcia et al. 2007).

1.2.2 pH and dissolved metals

West Coast streams affected by coal mining can have high acidity with pH as low as 2.68 (pers. obs.). pH is a measure of the amount and activity of free hydrogen ions in solution and is directly related to acidity which is the total excess of hydrogen ions over all other ions (Younger et al. 2002).

As acidity increases there is a correlated increase in the solvency of metals associated with coal and other minerals. Secondary minerals may be dissolved directly by hydrogen ions or may be catalytically dissolved by iron (III) ions, thereby increasing the metal load to the drainage (Plumlee et al. 1993). Depending on the type of coal and surrounding rock a number of toxic metals may find their way into solution. These include Fe, As, Mn, Cu, Al among many others (Moore et al. 2005).

1.3. Physical processes

1.3.1. Precipitate formation and deposition

Dissolved iron and other metals in receiving waters may precipitate as pH increases. This commonly occurs where the waterway is diluted through ground or surface waters entering the waterway or at a confluence with a less acidic stream. The two most common precipitates in AMD waterways are $FeOH_{3(s)}$ and $AlOH_{3(s)}$ which begin precipitating above pH 3.5 and 4.9 respectively. Where precipitate formation is rapid, waters may become turbid (Fig. 1.1.A) with the benthos becoming coated in a thick metal oxide layer. Metal oxide precipitates may clog and 'armour' the substrate, filling interstitial spaces.

Precipitate formation and its rate is dependant upon pH, temperature, metal species present and their concentrations. The latter both vary with pH (Kelly 1988; Younger et al. 2002; Jönsson et al. 2006). Metal oxide formation and deposition may start or the rate may dramatically increase where the pH is raised. If the pH of a waterway rises to circumneutral, the physical effects on the benthos may recover over a period of weeks (Niyogi et al. 1999).

1.3.2. Turbidity, Sedimentation

Opencast and alluvial mining also generates turbid runoff. During the process of open cast mining, vegetation is removed and soil and rock are exposed and processed. Fine particulate mineral matter enters surface waters increasing the turbidity and, dependant upon flow rates, the rate of sedimentation. Changes in the water velocity of a stream or river may lead to the deposition or resuspension of particulate material remobilizing contaminants into suspension (Kelly 1988).

Fine inorganic suspensoids or clays are understood to lead to extreme attenuation of light in stream water. They also significantly reduce dissolved oxygen, bed permeability and cause particle entrapment within the periphyton matrix (Davies-Colley et al. 1992; Quinn et al. 1992).

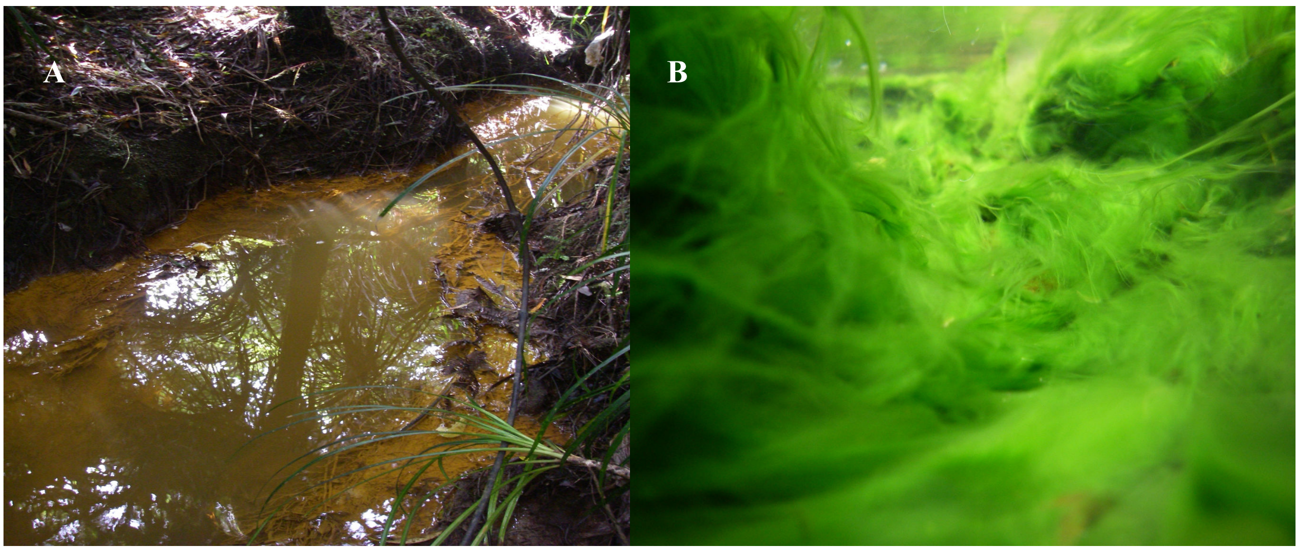

Figure 1.1. A, B. Two AMD impacted West Coast streams: A) West Coast Road Creek exhibiting such high rates of iron hydroxide deposition that no algae were found in this stream; B) Pack Track Stream (pH 2.9, conductivity 1220 μScm^{-1}), with prolific chlorophyte growths (*Klebsormidium acidophilum* dominated).

1.3.3. Sorption processes

Metal ions can adsorb onto mineral and organic surfaces. Where the surface is fixed the metal is immobilized. Where ions adsorb onto suspended organic matter they will remain mobile until the particles settle out. The adsorption of aqueous Fe(II) onto the iron oxide mineral surface is a rapid process and is followed by surface-catalyzed oxidation by dissolved O_2 leading to Fe(III) iron-hydroxide precipitate, causing further armoring of the substrate (Younger et al. 2002).

pH is a master variable for metal ion mobility, adsorption and precipitation which all affect bioavailability. There is a wide range of possible solute and adsorbed species and an increasing pH will tend to remove dissolved metals from discharges, while decreasing pH will act to keep them in solution. A drop in pH can also cause immobilized metals (sorbed and precipitated) to be remobilized from deposits within the benthos (Younger et al. 2002).

1.4. Acid mine drainage impacts on aquatic biota

1.4.1. Aquatic effects

AMD can affect aquatic ecosystems through a range of stressors (Figure 1). There are three major interacting stressors: (1) acidity, (2) heavy metals, (3) metal oxide deposition (Niyogi et al. 2002). Important factors affecting the degree of toxicity are metal speciation, acidity, activity, previous exposure (i.e. prior AMD pulse), and differences among species in their ability to regulate metal uptake (Anderson and Morel 1978; Yoshimura et al. 2000; Gross 2000). The sensitivity of algae to metals may also be dependant on nutrient concentrations. For example where communities are phosphorus limited they are more sensitive to copper, which may be especially pertinent to communities of oligotrophic streams (Guasch et al. 2004). Dissolved metals occur simultaneously in a number of different forms and each form has unique chemical properties and thus reactivity with regard to mobility, bioavailability and toxicity (Younger et al. 2002). Surface binding and uptake by metals decrease as pH decreases, and thus toxicity of metals may be relatively low at low pH sites, although this may depend on the particular metals dissolved in solution and active transport mechanisms (Gross 2000; Yoshimura et al. 2000).

Biological responses may include behavioral changes, reduced growth, health, and increased mortality (Brakke et al. 1992). The frequency of cell deformities may also be expected to increase as heavy metal contamination increases (McFarland et al. 1997). The age of a biofilm may moderate ecotoxicological effects, where extracellular products inhibit toxicity or physicochemical conditions change with biofilm depth (Jones et al. 2000), which has been demonstrated for metal contamination (Admiraal et al. 1999).

Whether AMD-induced substrate (metal oxide deposition) or aqueous effects (the toxicity of hydrogen ions and dissolved metals) have the most negative impacts on aquatic communities may depend on the characteristics of the ecosystem in question, the severity of each type of pollution and the tolerances of organisms present (Letterman and Mitsch 1978; McKnight and Feder 1984 cf. DeNicola and Stapleton 2002). Metal oxide and other inorganic matter deposition causes a reduction in interstitial spaces and benthic habitat heterogeneity, reducing refugia for both fish and invertebrates, and may smother algae or may provide a benthos unsuitable for colonization and growth (Anthony 1999; Niyogi 1999; Harding and Boothroyd 2004).

1.4.2. The fauna and the chemoheterotrophic microflora

AMD has been shown to reduce decomposition activities of bacteria and fungi (Burton et al. 1985; Johnson 1998; Batty and Younger *In press*), thereby degrading an extremely important component of lotic ecosystem functioning (Fisher and Likens 1973; Hynes 1975). AMD is known to detrimentally impact fish abundance and diversity (Letterman and Mitsch 1978) and numerous studies have noted that AMD streams have much lower invertebrate diversity and abundance dependant on the severity of pollution (Letterman and Mitsch 1978; Winterbourn and McDiffet 1996; Winterbourn et al. 2000; Harbrow 2001; Harding 2005). The hyporheos, i.e. the zone between surface waters and ground waters, is an important ecotone in lotic ecosystems. It is where the exchange of organisms, water, nutrients, oxygen, organic matter and other solutes occurs (Burrell and Scarsbrook 2004) and the diversity and abundance of its fauna is also detrimentally impacted by AMD (Anthony 1999).

1.4.3. Primary producers

Vascular plants are almost absent from streams affected by AMD (Fyson 2000) and have not been noted in New Zealand. Several species of bryophyte are known to occur in New Zealand AMD streams. The moss genera, *Blindia*, *Bryum*, *Sphagnum* and the liverworts *Jungermannia*, *Lopocolea* and an unidentified genus were noted by Winterbourn et al. (2000) and were shown to accumulate metals as is known to occur in other bryophytes (Engelman and McDiffet 1996).

Algae are a prominent feature of many AMD systems, tend to have very low diversity and are often dominated by few species (Tate et al. 1995; Verb and Vis 2000; Sabater et al.

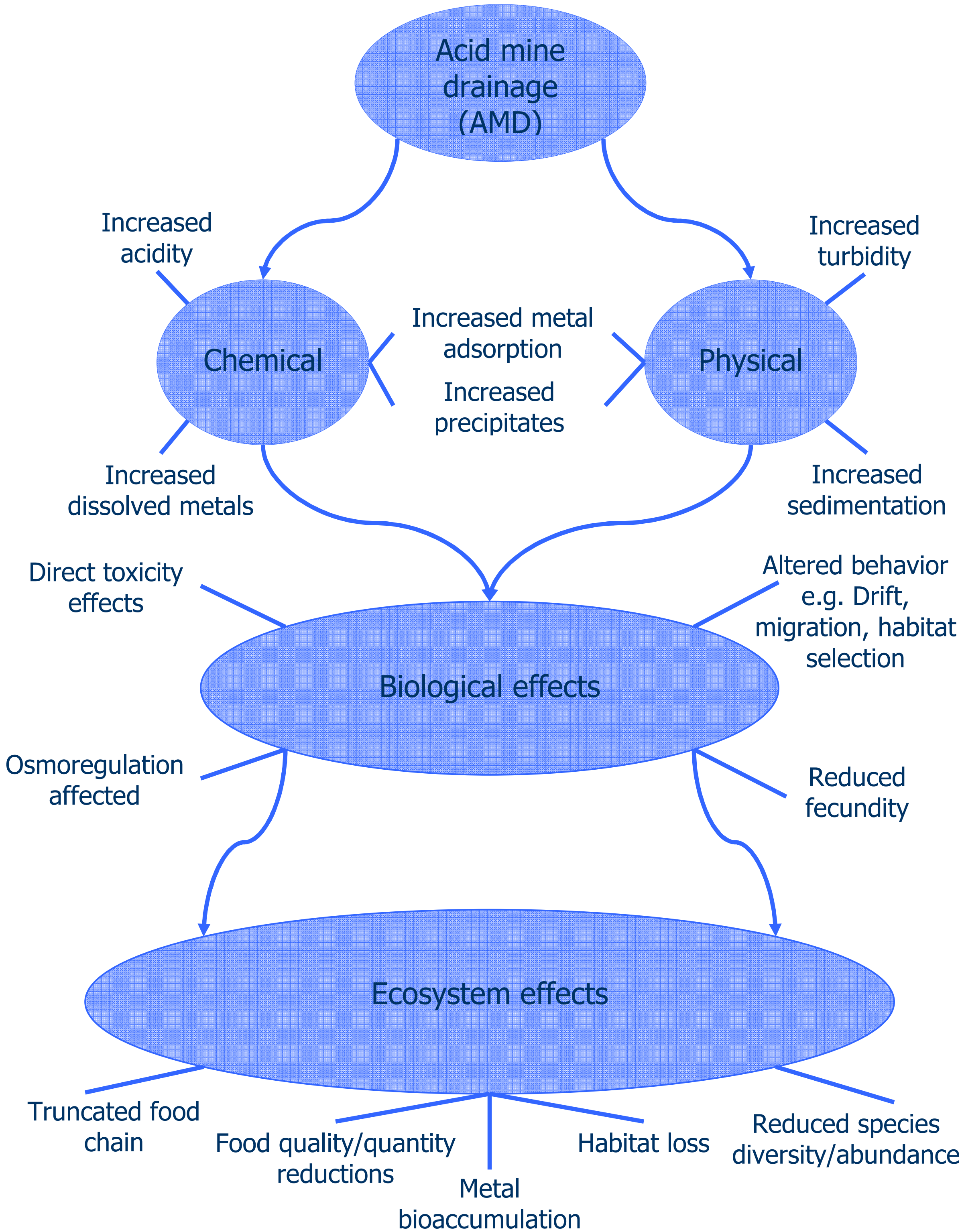

Figure 1.2. A model outlining the basic physical and chemical processes acting on acid mine drainage ecosystems (modified from Grey 1997).

2003), frequently with high biomass (Fig. 1.1.B; Brake 2001; Niyogi et al. 2002, Sabater et al. 2003) and productivity (Brake 2001). Some authors however, have found algal biomass to decrease at low pH (Kinross et al. 1993; Anthony 1999), while others have had conflicting results (Verb and Vis 2005).

Periphyton, benthic algae, *Aufwuchs*, microphytobenthos and biofilms all refer to the biota attached to submerged surfaces, while benthic algae and microphytobenthos refer specifically to the algal component of this submerged biofilm. Benthic algae may grow on sand (episammic), rock (epilithic), plants (epiphytic), mud or on silt (epipelic). The periphyton in AMD streams typically comprises a mixture of iron bacteria, fungal hyphae and algae with associated mineral particles (Sabater et al. 2003), but where periphyton is referred to in this document, it will be in particular reference to the algal component.

Acid mine drainage inputs have been shown to change the species structure of algal communities, where acidification causes many taxa to senesce while more tolerant taxa, e.g. *Ulothrix sp.*, establish or persist and become dominant (Niyogi et al. 1999). Certain authors have asserted that Cyanobacteria cannot grow below pH ~4 (John et al. 2002) and this is generally regarded as true, but Steinberg et al. (1998) have reported small-celled blue-green filaments resembling *Oscillatoria* sp. or *Limnothrix* sp. and *Spirulina* sp. from a Lusatian (Germany) acid lake of pH ~3.

A hypothesis has been proposed and tested to account for the effects of stress in aquatic ecosystems on diversity, biomass and function of benthic algae (Figure 1.3; Niyogi et al. 2002). The hypothesis suggests that biodiversity is very sensitive to change, while biomass and function (where function includes primary production, decomposition and nutrient cycling) may increase under low and moderate levels of stress only decreasing their response at much higher levels. Positive changes in function and biomass may occur for several reasons: 1) Tolerant species are stimulated, for instance where physicochemical conditions are, rather than acting as a stressor, optimized for a

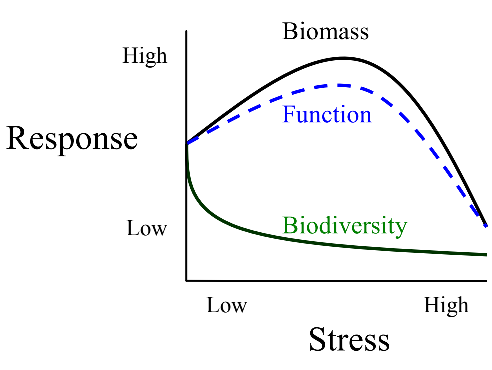

Figure 1.3. Hypothesized responses of primary producers to ecosystem stress (Niyogi et al 2002).

particular species, e.g. pH (Von Dach 1943; Olaveson and Nalewajko 2000); 2) competitors senesce due to altered physicochemical conditions releasing tolerant species from interspecific competition (Niyogi et al. 1999); 3) elevated metal concentrations may themselves act as a subsidy (Niyogi et al. 1999); and 4) physicochemical conditions exclude grazers thus releasing algae from any top-down control that would otherwise be occurring (Niyogi et al. 1999).

Niyogi et al. (2002) found this Biomass, Function, Biodiversity hypothesis applied for aqueous effects (pH, dissolved metals) but not for physical effects (precipitate deposition) where even a small level of metal oxide deposition decreased diversity, function and biomass. Similarly Anthony (1999) found algal biomass was low in most of the AMD-affected streams sampled (pH >4.5) on the West Coast, and suggested that precipitates may have prevented attachment of algae and precipitate adsorption onto algal cells may have inhibited photosynthesis. Other authors have found increased algal biomass at low pH, but where precipitate deposition remains low or is absent (Müller 1980; Stokes 1986; Mulholland et al. 1986; Niyogi et al. 1999).

Acidification changes algal community composition (Müller 1980; Hirst et al. 2004) as do aqueous heavy metals (Clements 1991; Medley and Clements 1998; Rousch and Sommerfeld 1999; Hill et al. 2000a; Soldo and Behra 2000). Soldo and Behra (2000) examined long-term effects of copper on a periphyton community and the short-term effects of copper, zinc, nickel and silver on communities and photosynthetic rate. During the long term experiment the original community changed from one dominated by Cyanophyta to one dominated by Chlorophyta. Only at the highest copper exposure was photosynthetic rate affected, otherwise there was no significant difference in photosynthesis between these two community types. They suggested a community previously exposed exhibited pollution-induced community tolerance (PICT), where a community exposed to a particular toxicant may be expected to display increased tolerance compared to a naive community. They also reported communities exposed to copper had co-tolerance to zinc, nickel and silver.

The most significant effect of AMD conditions on AMD tolerant communities is the deposition of metal oxides (McKnight and Feder 1984; Niyogi et al. 1999). Niyogi et al. (1999) observed an inverse relationship between deposition and biomass. They noted deposition rates to be high, in excess of 1.6 g $m^{-2}d^{-1}$, at a stream confluence but this steadily decreased with distance from the confluence and at 1 km was averaging ~0.6 g $m^{-2}d^{-1}$. Algal biomass was undetectable at high levels of deposition, while chlorophyll *a* concentrations reached ~ 80 mg m^{-2} at the lowest levels.

Niyogi et al. (1999) propose that aluminium oxide has a greater effect on periphyton abundance than iron oxide. They suggested that this may be because aluminium hydroxides are not subject to photochemical dissolution. Photochemical dissolution occurs where a particular compound is reduced in the presence of light, resulting in its dissolution. Iron hydroxides may settle onto algal filaments, e.g. *Ulothrix sp.* and because of this greater exposure to light (as opposed to the shaded substrate) they may be more readily photoreduced. Where photochemical dissolution occurs metal oxide deposition will present a lesser stress on growing periphyton (Tate et al. 1995; Niyogi et al. 1999).

Algae can modify the chemical and physical environment through removing pollutants via adsorption (Roy et al. 1993), absorption (Elbaz-Poulichet et al. 2000) and indirectly by modifying water chemistry (Das et al. 1991). Also, they have effects on conditions within the boundary layer, the layer of water adjacent to their cell surfaces, where for example the pH of water may increase by 1-2 units (Jones et al. 2000).

Some genera and species are common inhabitants of AMD habitats, for instance, *Euglena mutabilis* Schmidtz (Euglenophyceae, Euglenales) (Brake et al. 2001; Baker 2004), *Pinnularia acoricola* Hust. (Bacillariophyta, Pennatophyceae) and *Eunotia exigua* (Bréb. ex Kütz.) Rab. (Bacillariophyta, Eunotiales) (DeNicola 2000; Sabater et al. 2003). Lists of diatom taxa found in natural and polluted acidic habitats have been compiled by DeNicola (2000). Chlorophytes typically dominate AMD habitats, records include several species of *Klebsormidium* (Chlorophyta, Klebsormidiales), *Mougeotia sp*. (Chlorophyta, Zygnematales Ulotricales), *Zygnema sp*. (Chlorophyta, Zygnematales) and *Ulothrix sp*. (Chlorophyta, Ulotricales) among others (Bennet 1969; Verb and Vis 2001; Sabater et al. 2003). Several documented cases of *Klebsormidium* in AMD have identified at least two different forms, both *Klebsormidium flaccidum* Kützing and *Klebsormidium rivulare* Kützing (Verb and Vis 2000; Sabater et al. 2003). A recent study (Novis 2006) has identified a new species from AMD streams, *Klebsormidium acidophilum* Novis, using both molecular and morphological characterization. This species may best fit the *Klebsormidium* species and the *Ulothrix sp.*

recorded in numerous earlier studies of AMD waters (Niyogi et al. 1999; Winterbourn and McDiffet 2000; Novis 2006 and refs. therein).

Euglena mutabilis may be considered an acidophilic species and is possibly the most widely studied, observed and understood AMD alga (von Dach 1943; Bennet 1969; Warner 1971; Nakatsu and Hutchinson 1988; Lessman et al. 2000; Olaveson and Nalewajko 2000; Brake et al 2001; Sabater et al. 2003; Espana et al *In* press). It has also been studied in other low pH habitats, e.g. sulphur springs (Brake et al. 2001). This species is a facultative heterotroph capable of growth on soluble metabolites (Gross 2000) and is has been found in habitats with a pH as low as 1.7 and conductivities reaching 2800 μScm^{-1} (Brake et al. 2001). It can form thick mats and its photosynthetic activity has been noted to contribute to over-saturation of dissolved oxygen by up to 200%. *E. mutabilis* is often associated with an acidophilic yeast taxon with which it may have a mutualism (Nakatsu and Hutchinson 1988).

1.4.4. Algal adaptations for growth in AMD

Despite the harsh physicochemical conditions AMD imposes, a broad number of algae are adapted to growth and may be either acidophiles (limited to growth in acidic conditions) or acidotolerant (highly tolerant of acidic conditions). For algae that live in very low pH conditions several adaptations are necessary. A neutral cell pH must be maintained, which is made possible by a relatively impermeable plasma membrane, thus ensuring little energy is required for active transport across the gradient (Gross 2000).

Carbon is a primary resource for photosynthesis, but in acidic environments is quickly neutralized in mineral form. Most algae obtain C from atmospheric CO_2, but this requires movement to or growth in areas of high CO_2 availability. Due to the high CO_2 requirements and ambient shortages algae in acidic environments must have a CO_2 concentrating mechanism (Gross 2000).

Motility may be favored to best use environmental resources, light, CO_2, or to escape unfavorable conditions. Specific cell wall compounds such as algaenan, or possessing non-cellulosic cell walls may also facilitate growth and tolerance of low pH conditions. Extracellular enzymes like hydrolases (e.g. *E. mutabilis*) and phosphatases (e.g. *Chlamydomonas acidophila*) (Gross 2000) may be important where conditions limit resources or resource patches are encountered.

Chapter 2: Periphyton communities across a gradient of AMD stress

2.1. Introduction

Periphyton assemblages are complex and vary in time and space dependant on a wide range of environmental and biological factors (Biggs and Kilroy 2004; Peterson 2007). In freshwaters, light (DeNicola et al. 1992), nutrients (Biggs and Close 1989) temperature (DeNicola 1996), current velocity (Poff et al. 1990), physical disturbance (Biggs and Close 1989), competition (Peterson 2007) and invertebrate grazing (Peterson et al. 2001) are all significant factors affecting periphyton structure and productivity. Abiotic factors that control taxonomic composition of assemblages are defined at the catchment level of physical processes. Catchment geology, the origin of a waterway and the type of land use, define certain crucial factors influencing periphyton communities, such as flood frequency and nutrient loading (Biggs 1990), while other factors are more important at local scales (Biggs and Gerbeaux 1993).

AMD alters stream physicochemical conditions and its effects override other abiotic factors, becoming the primary conditions influencing periphyton community structure (Verb and Vis 2000; 2001; 2005), biomass and function (Niyogi et al. 2002). AMD can have a range of negative effects on algal communities, and these often occur due to three major abiotic factors. These are: high acidity, high concentrations of metal ions (e.g. Fe, Cu, Al), and metal oxide deposition, which is mostly $Fe(OH)_3$ in New Zealand (Niyogi et al. 2002, Harding and Boothroyd 2004). Algal communities may be structured by pH (Kinross et al. 1993; Hirst et al. 2004), heavy metal contamination (Soldo and Behra 2000) and metal oxide deposition (Niyogi et al. 1999) but AMD streams incorporate these factors (Niyogi et al. 2002). Establishing the relative roles of both anthropogenic and natural factors on communities, and further establishing the role of inherent community patchiness both spatial and temporal on the effects of these factors, is a persistent problem of biomonitoring studies (Clements and Kiffney 1995).

Algal studies in AMD systems have concentrated on the most severely affected habitats and their algal flora's (e.g. Brake et al. 2001; Sabater et al. 2003), or have had an emphasis on community function and biomass rather than composition (e.g. Niyogi et al. 1999, 2002). Verb and Vis (2001) however, investigated the macroalgal component of the AMD impacted Hocking River drainage basin, Ohio, USA. They reported lowered diversity and mixed results regarding standing crop as AMD impacts increased. They identified a community dominated by chlorophytes, and found that *Klebsormidium rivulare* and *Microspora tumidula* were indicative of AMD impacts. Other studies carried out on smaller spatial scales also indicated that chlorophytes dominated AMD and tolerant species of genera such as *Klebsormidium*, *Microspora*, *Mougeotia*, *Ulothrix, Stigeoclonium, Zygnema* and *Microthamnion* were common in severely affected streams (Warner 1971; Verb and Vis 2001; Stephens et al. 2001; Niyogi et al. 2002; Sabater et al. 2003; Verb and Vis 2005; Novis 2006).

Diatoms have also received much attention in AMD habitats. DeNicola (2000) produced a comprehensive review of diatoms in natural acidic habitats, such as sulphur ponds, and AMD. He identified 124 taxa in habitats of pH $\leq$3.5 from 28 different studies. Verb and Vis (2000) found the diatom flora to be predictable according to the level of AMD stress in Hocking River drainage basin, indicating they may be useful as bioindicators of this. Species of *Eunotia, Pinnularia*, *Achnanthes*, *Nitzschia*, *Cymbella*, *Fragilaria* and *Synedra* have all been reported from AMD habitats (McKnight and Feder 1984; Verb and Vis 2000; DeNicola 2000; Hill et al. 2000a; Sabater et al. 2003).

Surprisingly few authors have compared algal communities across a gradient of AMD impact, within or across catchments (Verb and Vis 2000; 2001; 2005). These studies have found predictable changes in species composition of diatom and total assemblages (Verb and Vis 2000; 2005) but not marked changes in the macroalgae (Verb and Vis 2001).

This chapter describes a broad scale survey of algal communities across a gradient of AMD contamination. The aims were to: 1) determine if differences in species composition of assemblages occurred across the AMD gradient, 2) establish whether AMD impacted algal diversity and assemblage dominance, 3) establish whether AMD affected algal cover and biomass, and 4) establish what major variables were responsible for observed differences.

2.2. Methods

2.2.1. Survey methods

A large scale survey was conducted of 52 streams encompassing a range of mine polluted states, from those severely affected by acid mine drainage to pristine streams unaffected by pollution sources. Each site was sampled on a single occasion between April 2006 and January 2007. Initially sites were selected from topographical maps in mining regions throughout the West Coast of New Zealand. At each site a 50 metre reach was selected that included at least one riffle, run, pool complex. Two transects were placed where algal cover was visually estimated to be at its highest within this reach.

At each transect ten estimates of algal cover and algal depth were recorded from each transect in a stratified manner (Verb and Vis 2001; Sabater et al. 2003). This was accomplished using a Perspex viewing tube specifically designed immersed to ~ 3cm from the substrate which had a viewable area of 160cm^2. Algal cover and the depth of growths could then be estimated for each visibly different macroscopic growth. Using the following equation an estimation of algal biomass was obtained for each growth type (Rodrigo 1995).

$$\text{estimated algal biomass} = \%\text{cover}*\text{depth(mm)}$$

A range of physicochemical measurements were taken at each site; including a channel stability index (Pfankuch 1975), mean surface water velocity, mean bed-width and mean channel depth. Depth and width were recorded at 5-10 intervals across the stream dependant upon stream width. Canopy cover was visually estimated from the centre of the reach and stream order and stream aspect were also estimated from topographical maps. The geographical location was recorded using a Handheld GPS (Garmin eTrex LegendC 2004). The predominant vegetation type was recorded and a substrate index was used to determine the relative area occupied by six substrate categories. Estimated percentages of each substrate category were multiplied by a weighted variable and summed giving a single continuous variable (Jowett and Richardson 1990).

The substrate index used to establish the type of substrate present at sites.

(substrate index) = (%bedrock*0.08) + (%boulder*0.07) + (%cobble*0.06) + (%gravel*0.05) + (%finegravel*0.04) + (%sand*0.03)

Spot measurements of pH, conductivity and temperature were recorded at each site using an Oakton pH/CON 10 Series field meter. The degree of precipitates present was estimated over the 50 m reach using a simple categorical index designed from observations made during a preliminary survey.

The following is the precipitate index used to estimate the relative degree of metal oxide deposition.

1 - No precipitates present.

2 - Low levels of precipitate deposition, deposition only apparent upon close inspection.

3 - Medium levels of precipitate deposition, around 25-50% of the substrate has immediately visible deposition, while other areas are clear.

4 - High levels of precipitates present, much of the substrate 50-75% is covered, with some cementing. Low levels of turbidity.

5 - Very high levels of precipitates present, 75-100% of the substrate armored and cemented with high levels of active deposition occurring, indicated by high levels of turbidity.

At each site notes were made on visible algal growth and descriptions of each site and journey waypoints were also recorded to enable the site to be easily revisited. Each macroscopically different growth recorded, was collected for analysis of taxonomic composition and the relative abundance of taxa present was estimated. All different growth forms observed were collected. These were placed on ice and where possible were refrigerated until they were transported to the laboratory for preservation and analysis. Algae collection methods included whole rock sampling, cobble and gravel scraping. Where it was necessary to sample boulders or bedrock a simple modified bedrock sampler was employed. Algal collection methods followed those of Biggs and Kilroy (2000).

In the laboratory a sub-sample of each ‘macroscopically visible growth form’ was added to ~100mls of tap water. This was homogenized using a handheld blender for 15s.

This homogenate was then preserved in Lugols iodine and stored before establishing for each sample the taxonomic composition and the relative abundance of taxa present.

A sub-sample of each growth form was kept in a temperature controlled incubator for identifications. Relative abundance counts were carried out on preserved samples and were made using one to three slides of sample homogenate and counting over 300 'algal units'. Algal units were individual algal cells or a fragment of a larger body and were counted at 400-1000X magnification. Diatoms were counted only where they had a visible chloroplast, degraded or not. An Olympus BX50 was used for identifications and an Olympus Camedia C5060 Wide Zoom digital cameral for photographic records. Identifications were made to the lowest taxonomic unit, to genus and often to species (Foged 1979; Krammer and Lange-Bertalot 1991a; 1991b; 1997; Biggs and Kilroy 2000; Lange-Bertalot 2001; John et al. 2002; Novis 2004; 2006).

A number of indices were calculated including Simpson's diversity index and the Berger-Parker index of dominance. Simpson's diversity index (D) takes into account diversity and the equitability or evenness of species and was calculated, for each site, from the complete data set, using the following equation (DeJong 1975):

$$D = \frac{\Sigma n(n - 1)}{N(N - 1)}$$

(n) is the total number of a particular species.
(N) is the total number of organisms of all species.

The Berger-Parker dominance index (d) is a diversity index used for ascertaining the dominance of organisms within a sample and was calculated using the following equation (Southwood 1966):

$$d = N_{max} / NT$$

(N_{max}) is the number of individuals in the most abundant taxonomic group.
(NT) is the total number of individuals.

2.2.2. Study sites

Five regions containing a total of 52 sites were sampled on the West Coast of New Zealand. Three sites were in the vicinity of Blackball, eight near Greymouth, 23 near Reefton and 18 near Westport. Sites were selected from topographical maps (MetaMedia Ltd. 2000) based on their accessibility and whether they received AMD (AMD sites) or not (reference sites). Mapping was carried out using ArcGIS 9.2 (ESRI 2006).

2.2.3. Data analyses

Both PC-ORD Version 4.01 (McCune and Mefford 1999) and STATISTICA Version 7.1 (StatSoft Inc. 2006) were used for the statistical analysis of data. Graphing was primarily carried out using SigmaPlot Version 9.01 (Systat Software Inc. 2004)

A hierarchical cluster analysis was performed to establish AMD 'impact categories'. Site groupings from this analysis were used for comparisons of biological data (McCune and Mefford 1999).

Canonical correspondence analyses (CCA) were run to establish which if any of the environmental variables influence community composition. CCA seeks structure in the species matrix, while maximizing the relationship strength with the environmental matrix. It was also used to identify species strongly associated with impact groups or physicochemical parameters. TWINSPAN was also used to assist in the identification of these species (analysis not shown; McCune and Mefford 1999)

Single factor ANOVA's and both non-parametric and parametric correlation analyses were conducted to establish relationships within environmental variables and between environmental variables and the biological data. Where parametric tests were employed using environmental data, the data was transformed to meet normality. Normality was tested using Kolmogorov-Smirnov and Lilliefors tests, and was visually assessed using histograms generated using STATISTICA (StatSoft Inc. 2006). Residual scatter plots were used to ensure the variances were not heteroscedastic.

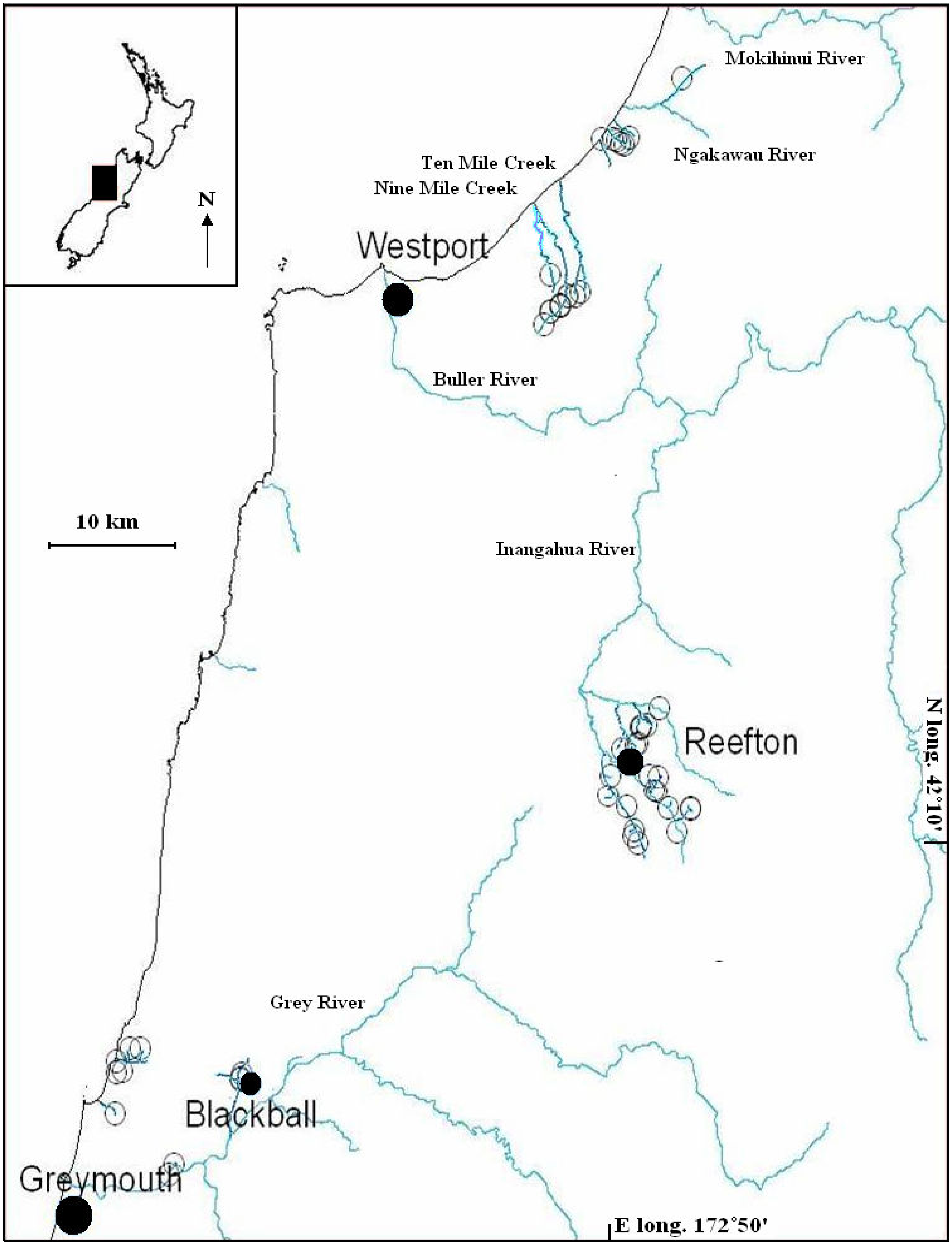

Figure 2.1. Map of the 52 survey sites sampled from April 2006 to January 2007.

2.3. Results

2.3.1. Categorization of sites

Across the AMD gradient sampled, pH values ranged from 2.7 to 7.6, conductivities from 17.9 to 1220 μ S cm^{-1}. Iron hydroxide deposition was absent in many of the sites and in a select few was so severe that little if anything was alive. The site named Coast Road Creek had such heavy iron oxide deposition that algae were absent and therefore the site could not be used in many of the following analyses. Sites had varying physicochemical characteristics (Appendix 1).

Sites were classified into four water chemistry groups based on AMD contamination variables using a hierarchical cluster analysis. A total of four AMD categories were chosen (Fig. 2.3.), where the category titles are ‘Reference’ including some marginally impacted streams, ‘Mild’, ‘Moderate’ and ‘Severe’. The variables analyzed were pH, conductivity as untransformed continuous variates and precipitates as a categorical variable. AMD impact categories differed significantly between pH, precipitates, conductivity and temperature (ANOVA Table 2.1.). Severely impacted sites had very low pH values, very high conductivities and high levels of estimated precipitate deposition, moderately impacted sites had higher but variable pH values, lower conductivities and lower estimated levels of precipitate deposition. Mildly impacted streams again had variable pH’s (mean 5.5) and relatively high conductivities. Reference sites again had variable pH but all had very low conductivities and generally had no observed metal oxide deposition. Conductivity was found to be the major structuring variate used by the Cluster analysis and was highly significantly different between all levels of the impact categories (Tukeys HSD test, $P < 0.000$).

The precipitate index differed significantly between reference sites and both moderately (Tukeys HSD test, $P = 0.012$) and severely impacted sites (Tukeys HSD test, $P = 0.0088$; Table 2). The pH in severely affected streams was significantly lower than reference, mild and moderately impacted streams (Tukeys HSD test, $P = 0.0015$). While temperature was significantly higher in severely affected sites compared to both moderate (Tukeys HSD test, $P = 0.034$) and mild streams (Tukeys HSD test, $P = 0.005$; Table 2.1.).

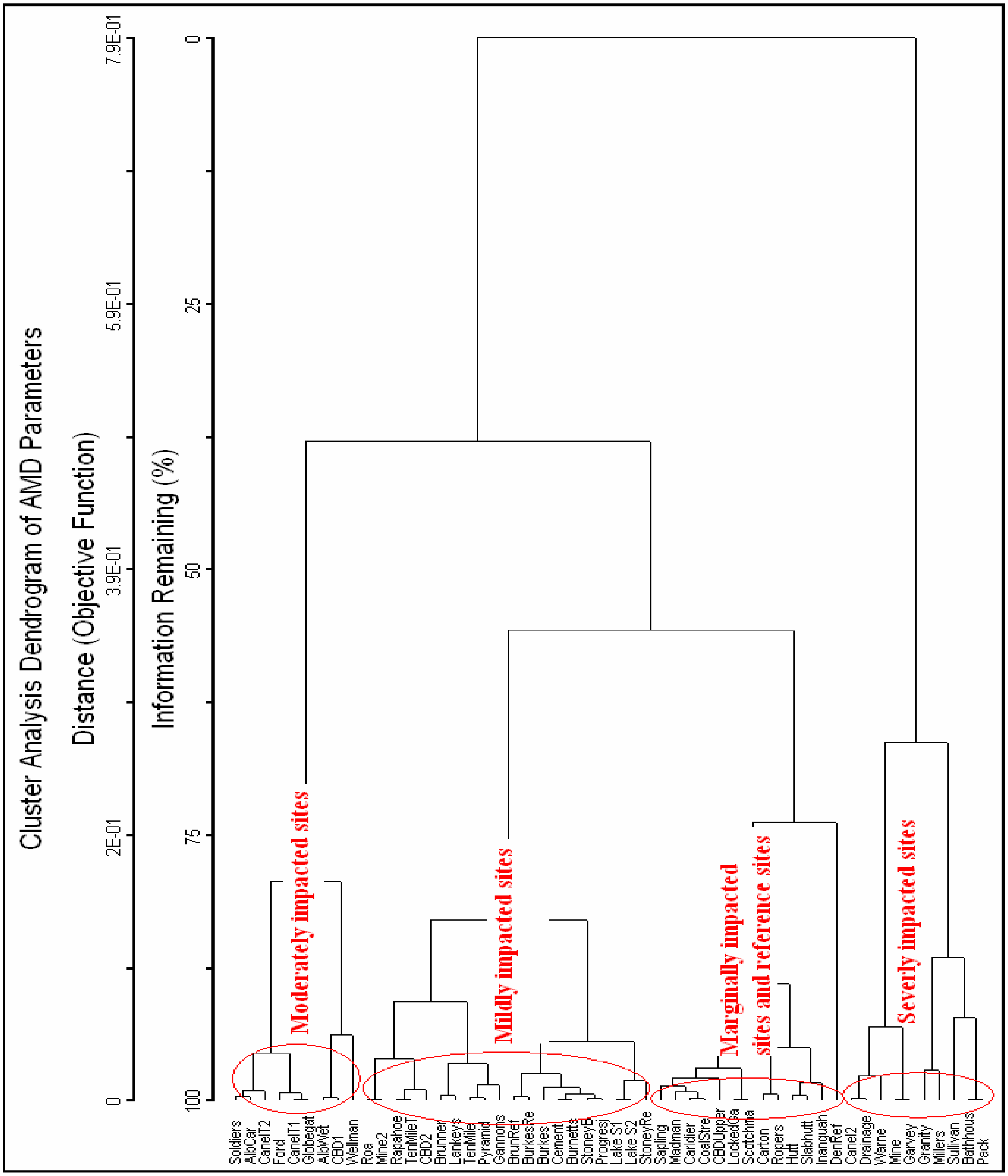

Figure 2.2. Cluster analysis of water chemistry (ph, conductivity, level of precipitates) of 52 sites sampled from April 2006 to January 2007. The linkage method used was group average and the distance measure was Bray-Curtis similarity. Percent chaining was calculated at 3.90.

Table 2.1. Single factor ANOVA statistics of key water chemistry and physical variables between the four AMD categories (mean values shown with ± SE, significance at P<0.05).

	Reference n = 13	Mild n = 20	Moderate n = 9	Severe n = 10	F	P
pH	6.3(0.3)	5.5(0.4)	5.5(0.3)	3.2(0.1)	$12.7_{df=3}$	**0.000**
Conductivity μScm^{-1}	33(37)	83(30)	196(45)	735(43)	$220.5_{df=3}$	**0.000**
Precipitates	1.2(0.2)	2(0.3)	3(0.6)	3(0.4)	$5.3_{df=3}$	**0.003**
Temperature ºC	11.2(0.6)	11.1(0.5)	10.0(0.7)	13.3(0.7)	$4.6_{df=3}$	**0.007**
Pfankuch	60(3.1)	60(2.5)	58 (3.8)	57 (3.6)	$0.9_{df=3}$	NS
Surface velocity (mS^{-1})	0.4(0.1)	0.6(0.08)	0.4(0.1)	0.6(0.1)	$0.8_{df=3}$	NS
Canopy cover (%)	32(6.2)	28(5)	42(7.4)	39(7.1)	$1.2_{df=3}$	NS
Substrate	5.3(0.4)	6.0(0.3)	5.6(0.4)	5.5(0.4)	$0.8_{df=3}$	NS
Width (m)	0.4(0.1)	0.6(0.07)	0.6(0.1)	0.6(0.1)	$0.7_{df=3}$	NS
Depth (m)	0.1(0.02)	0.2(0.02)	0.1(0.02)	0.1(0.02)	$0.3_{df=3}$	NS

2.3.2. Non AMD environmental parameters

Principle components analysis of non-contamination abiotic data revealed all but two sites were very similar with regard to these variables (graph not shown). The characteristics measured for this analysis were depth, stream width, surface water velocity, temperature, canopy cover and the stream stability and the substrate index. The two sites observed to differ in these variables were Ten Mile Creek, the largest waterway sampled and the site named Alborn Wetland Stream, which had almost lentic conditions

2.3.3. Periphyton communities

Total taxonomic richness was markedly higher in Reference and Mildly impacted streams than moderate and severely impacted streams. Severely impacted streams had much lower taxonomic richness where only 15 taxa were noted. Mean taxonomic richness, Simpson's diversity index and the Berger-Parker index of dominance however, do not change significantly with regard to the level of AMD stress. Algal cover and algal biomass estimates do change significantly, where severely affected sites have significantly greater levels than reference, mildly and moderately impacted streams (Table 2.2.).

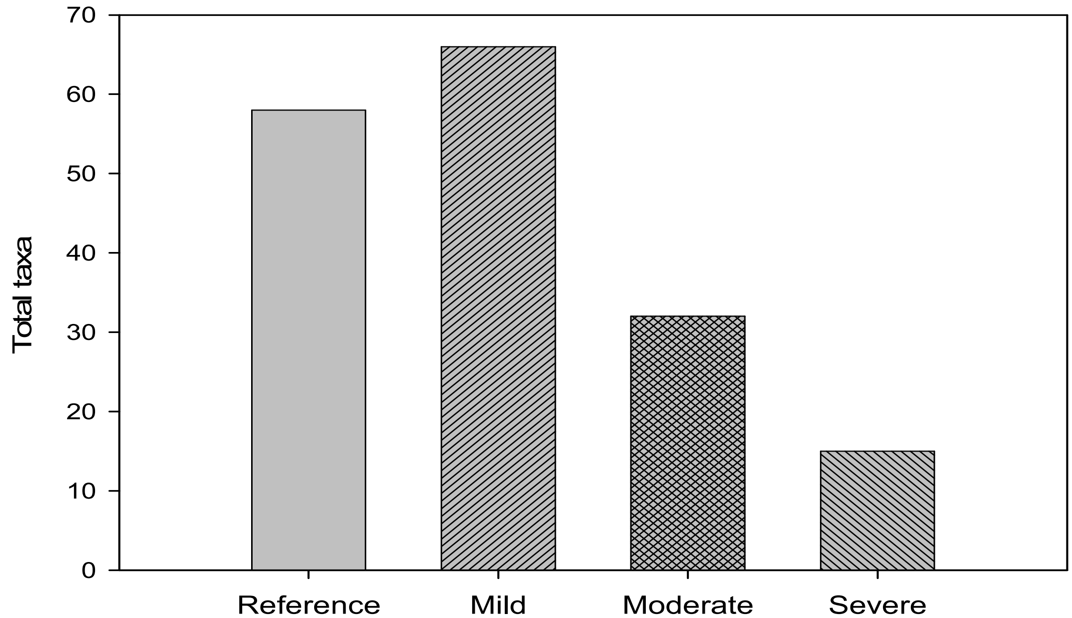

Figure 2.3. Total taxonomic richness in all sites of each of the four AMD categories (Severe n=10; Moderate n=9; Mild n=20; Reference n=13).

Table 2.2. Results of single factor ANOVA testing between selected biological metrics and AMD categories. (mean values shown with ± SE, significance at P<0.05).

	Reference n = 13	Mild n = 20	Moderate n = 9	Severe n = 10	F	P
Algal cover	15.9(7.2)	17.7(5.77)	18.2(8.6)	46.9(8.2)	3.6	**0.021**
Biomass index	36.2(62.3)	64.4(50.20)	18.6(74.8)	297.9(71.0)	3.49	**0.023**
Berger-Parker index	0.6(0.06)	0.6(0.05)	0.6(0.07)	0.7(0.07)	0.85	NS
Simpson's index	0.5(0.07)	0.5(0.06)	0.4(0.08)	0.6(0.08)	1.04	NS
Taxonomic richness	7.5(0.9)	6.1(0.76)	5.8(1.1)	4.6(1.07)	1.46	NS

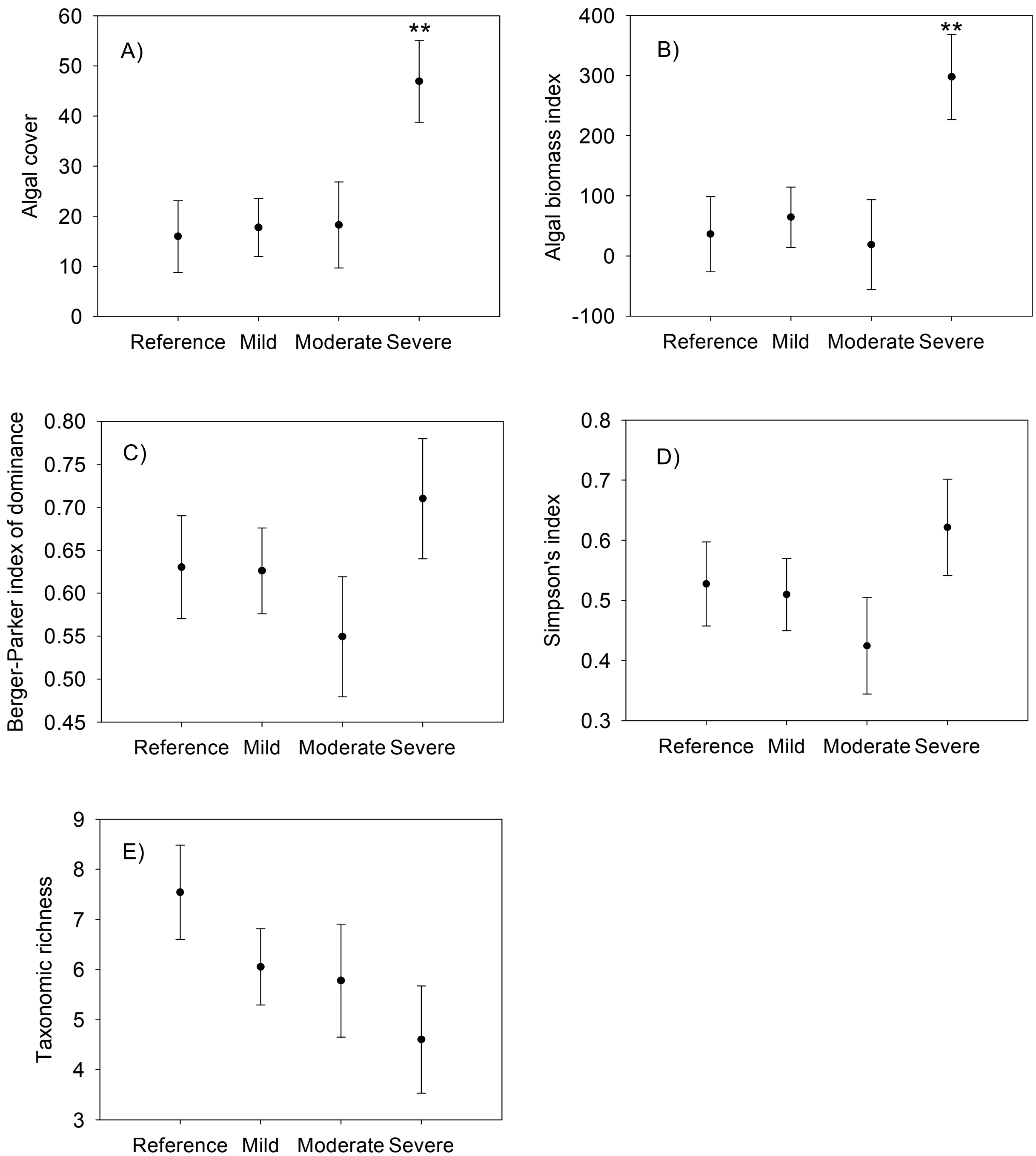

Figure 2.4. (A-E). Selected biotic indices: A) algal cover ($F=3.6_{df=3}$, P=0.021), B) algal biomass index ($F=3.49_{df=3}$, P=0.023), C) Berger-Parker index of dominance, D) Simpson's index and E) taxonomic richness compared with impact categories: severe (n=13), moderate (n=20), mild (n=9), reference (n=10) (mean values shown with ± SE, significance at P<0.05 **).

The structure of algal phyla also differed dependant on the degree of AMD impact (Fig. 2.5). Severely impacted streams were dominated by chlorophytes which accounted for 63% of the community. Euglenophytes (euglenoids) were represented by just *Euglena mutabilis* (25%) and bacillariophytes (diatoms) (15%), which was often dominated by *Navicula cincta*. Cyanophytes (blue-green algae) appeared in moderately impacted streams where they make up 27% of community structure and retain a similar abundance in both mild and reference streams. Rhodophytes (red algae) appear (0.6%) in moderately impacted streams, and their relative abundance increased steadily between mildly impacted (15%) and reference streams (27%).

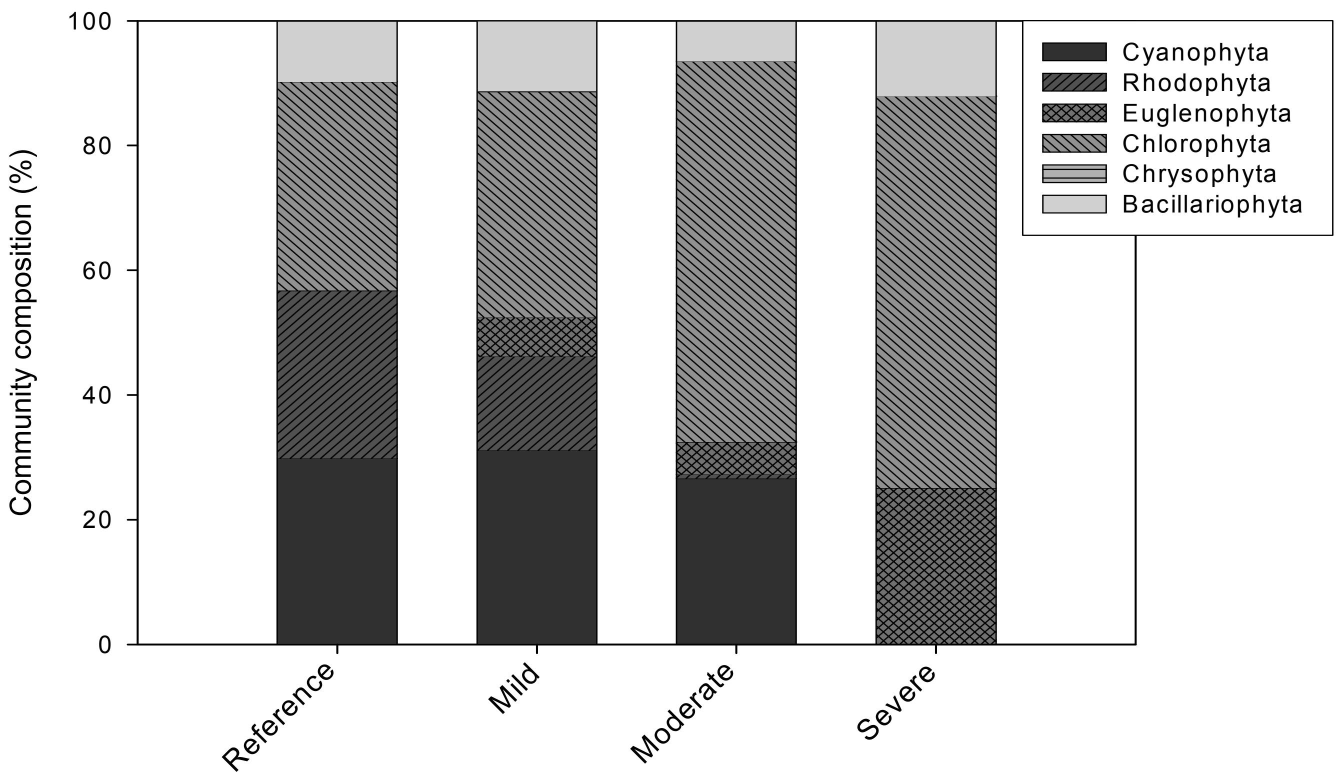

Figure 2.5. Differences in the relative abundance of phyla by the level of AMD impact. (Severe n=13; Moderate n=20; Mild n=9; Reference n=10).

E. mutabilis decreased in relative abundance as water quality improved and was completely absent from reference streams. Chlorophytes were dominant throughout all stress categories however less so in mild and reference streams. The only xanthophycean positively identified was *Tribonema* sp. and represented 0.1% in mildly impacted streams, while the percent composition of bacillariophytes varied little between categories.

The relative abundance of the five dominant phyla varied according to pH (Fig. 2.6). Bacillariophytes were present throughout the entire pH range while chlorophytes dominate across much of the pH range accounting for up to 100% of community composition at some sites. At circumneutral pH the relative composition of chlorophytes decreases, where cyanophytes become more important. Rhodophytes have a more restricted pH range and rarely dominated community composition. The sole Euglenophyte *Euglena mutabilis* was often abundant in AMD habitats where on several occasions it made up more than 60% of community composition.

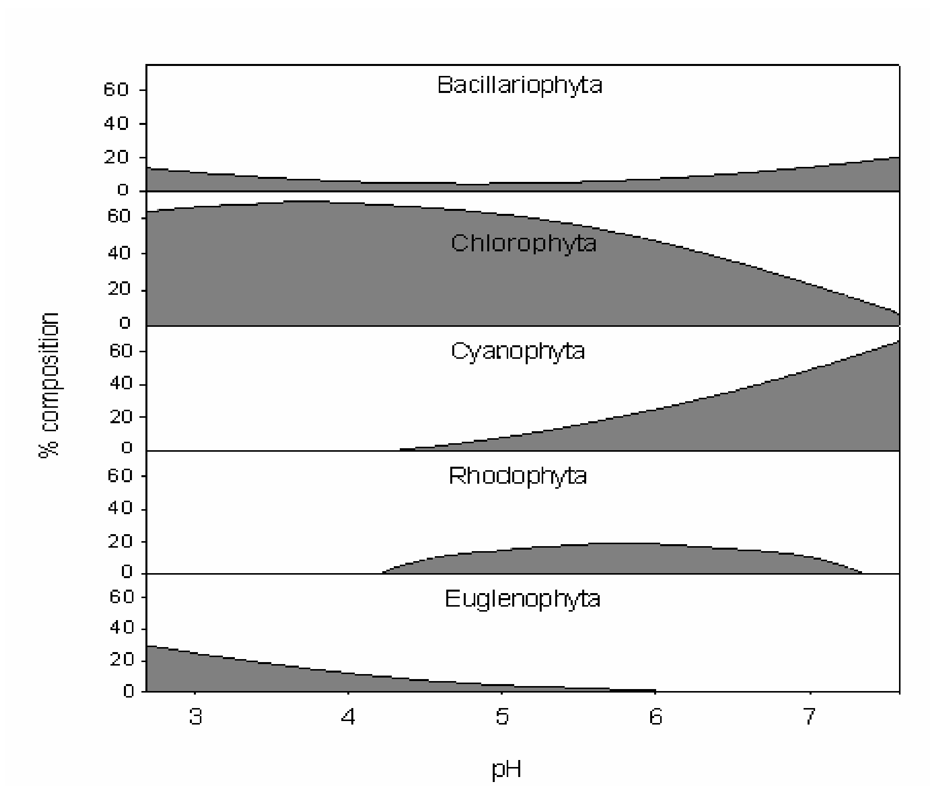

Figure 2.6. The relationship between the dominance of phyla and pH. The pH scale ranges from 2.7-7.6.

2.3.4. Environmental factors influencing communities

The Canonical correspondence analysis (CCA) of the entire relative abundance community data revealed several environmental variables were closely correlated with the species matrix (Fig. 2.7.). Sites are generally spread along Axis 1, which was closely correlated with pH (0.95) and also correlated with conductivity (-0.62), the precipitate index (-0.42) and temperature (-0.53). Axis two was primarily correlated with the precipitate index (-0.75) and depth (0.58). Severely impacted sites were clumped to the left of axis one. Reference streams were grouped to the right of axis 1 and moderate and mildly impacted streams are scattered along this axis. The precipitate index was also an important factor influencing the structure of some communities, which occurred towards the bottom of

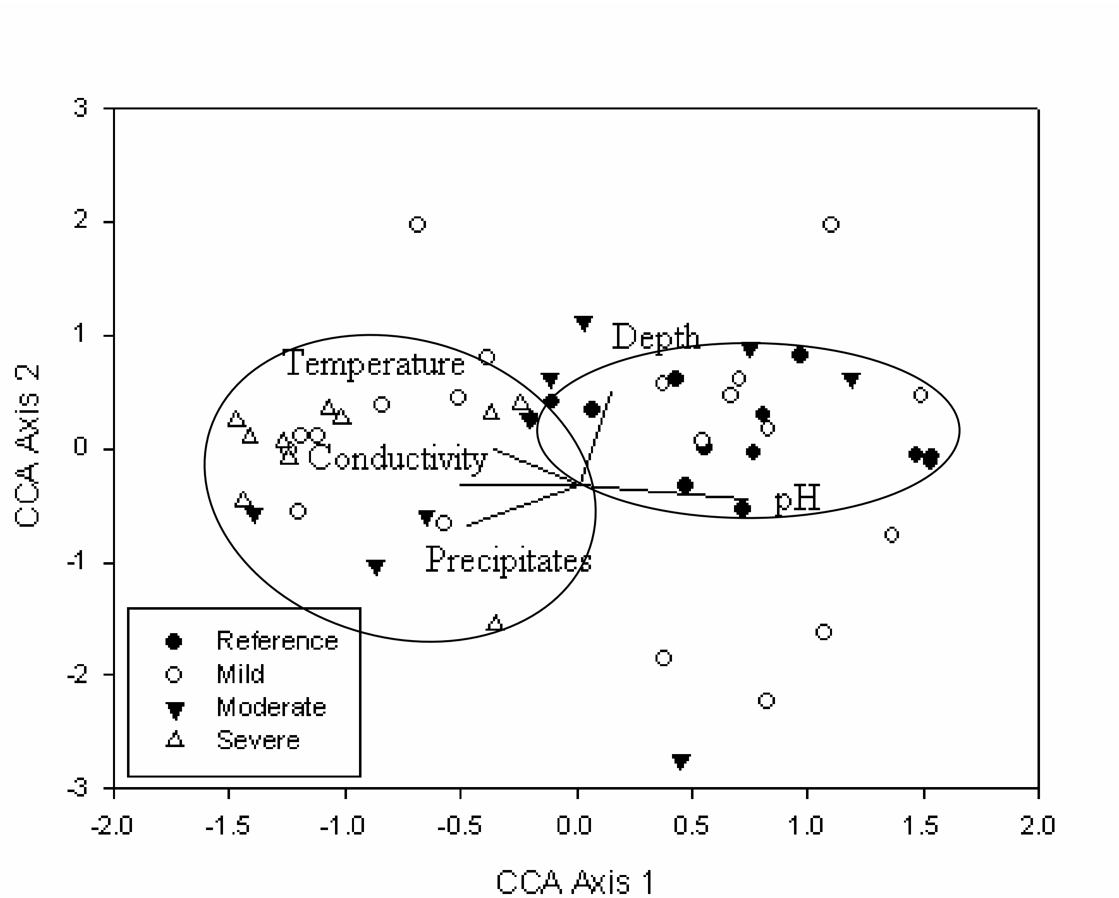

Figure 2.7. CCA showing the relationship between the environmental variables and algal assemblages at each site (n=52).

Axis 2. The analysis was stable with very low Monte Carlo correlation proportions, and had high species-environment Pearson correlations r = 0.94 and r = 0.92 for axes 1 and 2 respectively. Approximately 10% of the variation in the species matrix was explained by the environmental matrix (Table 2.3).

A CCA of the diatom community clearly separates reference and severely affected AMD sites (Fig. 2.8). The variables pH (-0.90), conductivity (-0.78), temperature (0.73), geographic location (-0.76) and season (-0.58) are key variables explaining community variance along axis 1. While precipitates (-0.56), temperature (-0.40) and season (0.52) explain community variance along axis 2 (correlation coefficients are ter Braak correlations 1986; Table 2.3).Approximately 26% of the variation in the diatom community matrix is explained when it is correlated with the environmental matrix.

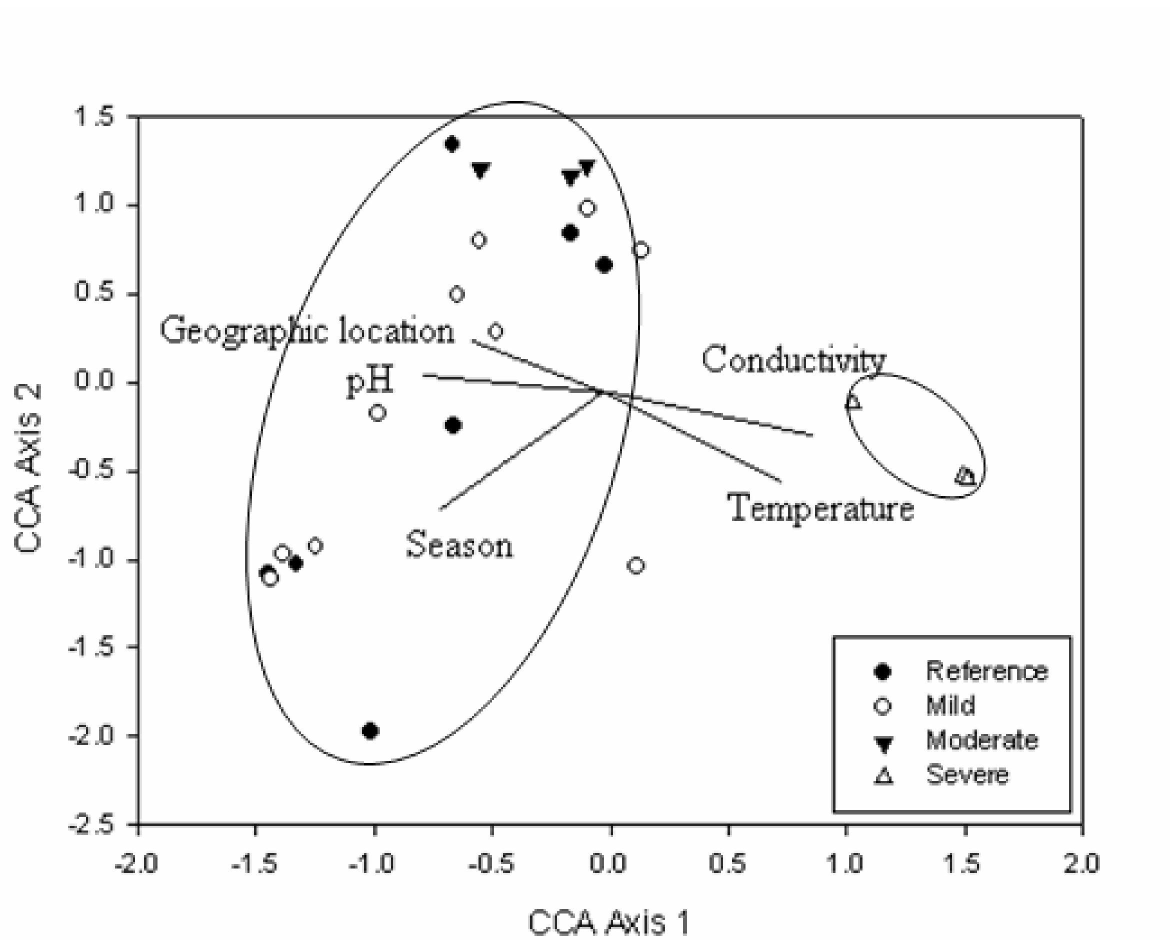

Figure 2.8. CCA showing the relationship between the environmental variables collected and sites based on diatom assemblages (n= 27).

Table 2.3. Canonical correspondence analysis statistics and axis correlations (ter Braak 1986)

	Complete assemblage CCA			Macroalgal CCA			Diatom CCA		
CCA Axes	1	2	3	1	2	3	1	2	3
Eigenvalue	0.75	0.64	0.61	0.79	0.60	0.57	0.98	0.95	0.87
Cumulative variance explained (%)	3.80	7.00	10.00	4.90	8.70	12.30	9.00	17.80	25.90
Pearson correlation (Spp-Evnt)	0.94	0.92	0.92	0.94	0.86	0.89	0.99	0.99	0.97
Axis correlations									
pH	0.95	-0.02	-0.05	-0.83	0.42	-0.07	-0.90	0.10	0.02
Conductivity	-0.62	-0.01	0.03	0.52	-0.22	0.12	0.78	-0.21	0.17
Precipitates	-0.46	-0.75	-0.22	0.70	0.05	-0.25	-0.03	0.56	0.22
Temperate	-0.53	0.28	0.15	0.38	-0.21	-0.01	0.73	-0.40	-0.13
Pfankuch	0.19	-0.07	-0.06	-0.12	0.25	-0.23	-0.25	-0.21	-0.32
Surface velocity	-0.24	0.22	0.19	0.24	-0.04	0.07	0.18	-0.20	0.42
Canopy cover	0.13	-0.06	0.15	-0.27	0.31	-0.33	0.12	0.26	-0.39
Substrate	-0.08	0.28	0.02	0.11	-0.11	0.14	0.17	0.33	0.30
Width	0.24	0.41	-0.21	-0.47	-0.40	-0.05	-0.04	0.20	0.53
Depth	0.24	0.58	-0.08	-0.63	-0.57	0.06	0.24	-0.17	0.56
Month	0.38	-0.32	0.69	-0.24	0.36	0.15	-0.76	-0.52	0.06
Geographic location	0.38	0.15	-0.06	-0.42	-0.11	-0.53	-0.58	0.26	-0.21

Algal cover and biomass increase significantly as conductivity increases (r=0.41, P=0.003; r=0.38, P=0.005) and pH decreases (r = 0.48, P<0.000; r=0.40, P=0.003) and as conductivity and acidity increase related to the degree of AMD impact, the relative level of precipitate deposition also increases.

Where precipitate deposition was estimated to be at its greatest algal cover (ANOVA, $F=8.1_{df=4}$, P=0.000) and biomass (ANOVA, $F=5.1_{df=4}$, P=0.002) are detrimentally impacted to the point where both are essentially absent (Fig. 2.9.). With increasing levels of precipitates richness begins declining, until richness is close to 0 where precipitate deposition was estimated to be at its highest (ANOVA, $F= 4.423_{df=4}$, P=0.004; Fig. 2.9.). Many taxa showed relationships with pH, conductivity and the relative level of precipitates (Fig. 2.10.).

Some showed distinct preference for AMD impacted sites. *Navicula cincta*, *Klebsormidium acidophilum*, *Microthamnion kuetzingianum* and *Euglena mutabilis* were all significantly positively correlated with decreasing pH, increasing conductivity and precipitates.

In contrast, *Microspora* cf. *quadrata* Hazen, occurred across broad pH (2.9 - 6.9) and conductivity (24-1200 μ S cm^{-1}) ranges, preferred low pH but had no obvious preference for conductivity. Many algae showed no significant relationship with pH, conductivity or the estimated level of precipitates, for example *Zygnema* cf. *cylindrospermum*, despite the fact it was most abundant in Wellman Creek a moderately impacted site that had a pH 4.6 and a conductivity of 300 μ S cm^{-1}. Other taxa, e.g. *Chamaesiphon* cf. *incrustans*, *Heteroleibleinia purpurascens* and *Cymbella kappi* were all negatively associated with increasing levels of each of the pollution variables.

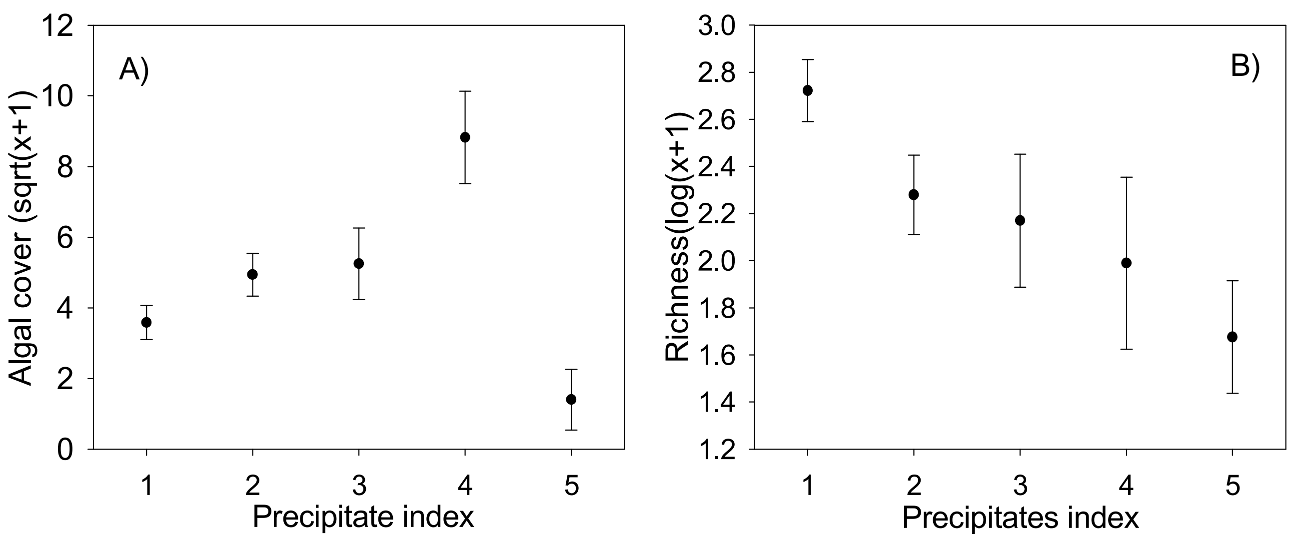

Figure 2.9. Relationship between the precipitate index and A) algal cover (ANOVA, $F=8.1_{df=4}$, P=0.000) and B) taxonomic richness (ANOVA, $F= 4.37_{df=4}$, P=0.004) (mean values shown with ± SE).

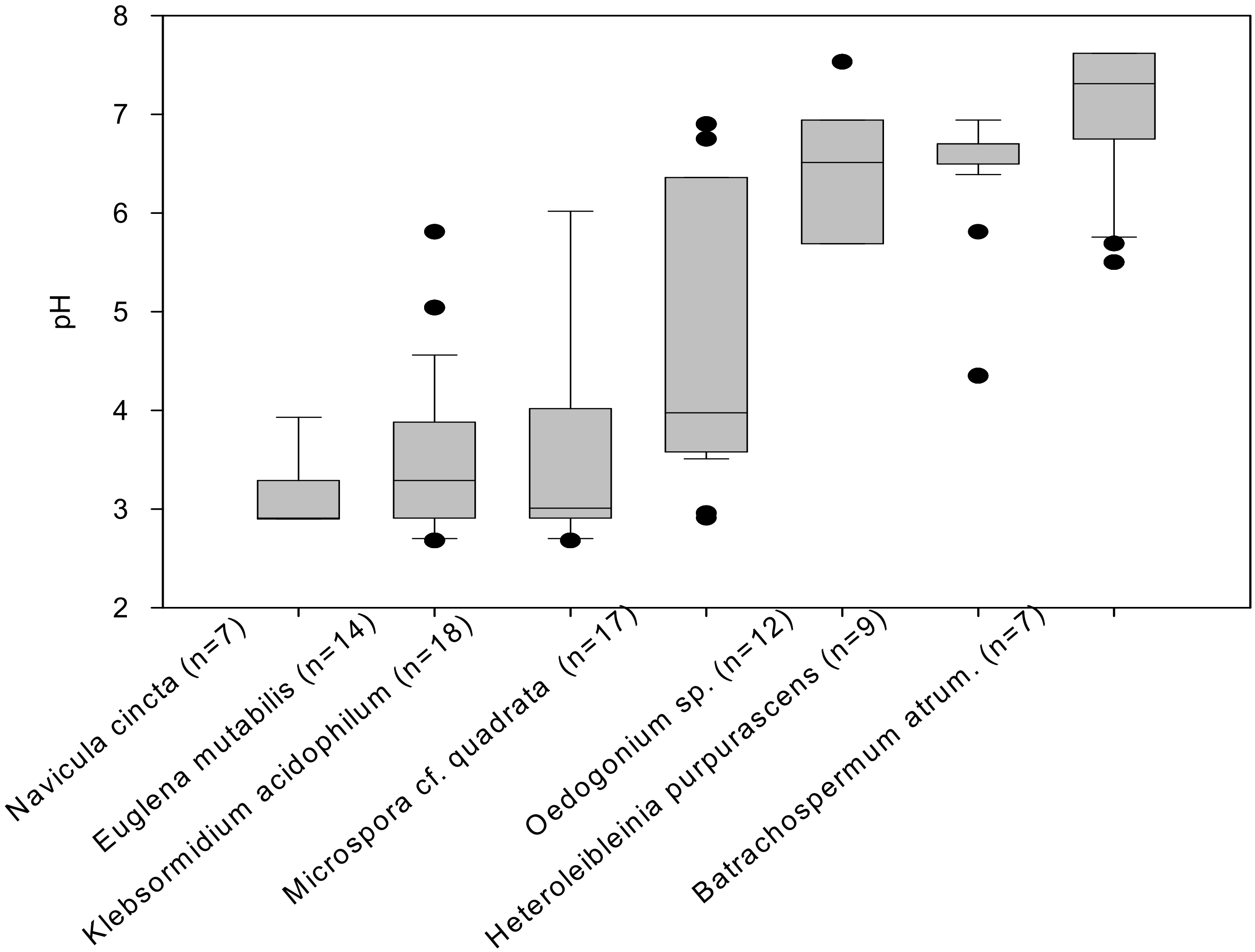

Figure 2.10. Relationships between pH and the abundance of seven dominant taxa. The upper and lower limits of the box represent the 75th and 25th percentiles respectively, the dividing line, the median and the vertical bars 90th and 10th percentiles. Outliers are included as dots.

Table 2.4. Spearman rank order correlations of the relative abundance of dominant taxa (≥ 5 occurrences) with pH, conductivity (C) and the precipitate index (PI). Significant statistics (P<0.05) are in bold type.

Phyla	Taxa (n)	pH	C	PI
Cyanophyta	*Chamaesiphon cf. incrustans* Grunow (5)	**0.36**	**-0.33**	**-0.33**
	Gloeocapsa sp. (5)	0.14	-0.14	-0.25
	Heteroleibleinia purpurascens (Hansgirg ex Hansgirg) Anagnostidis & Komárek (9)	**0.43**	**-0.46**	**-0.47**
	Pseudanabaena sp. (6)	0.20	0.02	-0.12
Rhodophyta	*Batrachospermum atrum* (Hudson) Harvey (7)	0.24	**-0.29**	**-0.33**
	Batrachospermum 'chantransia' stage (9)	0.25	-0.22	-0.22
Euglenophyta	*Euglena mutabilis* Schmidtz (14)	**-0.58**	**0.55**	**0.34**
Chlorophyta	*Characium* sp. (5)	-0.24	0.22	0.07
	Klebsormidium acidophilum Novis (18)	**-0.70**	**0.62**	**0.52**
	Klebsormidium rivulare (Kützing) Morison et. Sheath (12)	0.24	-0.26	**-0.36**
	Microspora quadrata Hazen (17)	**-0.30**	0.19	0.07
	Microthamnion kuetzingianum Nägeli (14)	**-0.34**	**0.41**	**0.32**
	Mougeotia cf. *depressa* (Hassal) Whittrock (6)	-0.26	0.13	0.15
	Mougeotia cf. *laevis* (Kützing) Archer (13)	**-0.30**	0.02	-0.07
	Oedogonium sp. (12)	**0.45**	-0.17	**-0.40**
	Zygnema cf. *cylindrospermum* (West et. G.S. West) Krieger (7)	-0.27	0.12	0.09
Bacillariophyta	*Cymbella kappi* Cholnoky (6)	**0.47**	**-0.29**	**-0.37**
	Frustulia rhomboides var. *crassinerva* (Brébisson) Ross. (5)	**0.28**	-0.08	0.03
	Gomphonema parvulum (Kütz) Grun. (5)	**0.39**	-0.27	-0.21
	Navicula cincta (Ehrenberg) Ralfs (7)	**-0.47**	**0.33**	0.15
	Navicula capitoradiata Germain (5)	0.13	0.03	-0.17

2.3.5. Comparing algal communities across a gradient of AMD stress

This study found a number of algae capable of tolerating the conditions of severely affected streams (12 taxa). Chlorophytes dominated and were represented by an unidentified unicellular chlorophyte, a species of *Gloeocystis*, *Microspora* cf. *quadrata,, Microthamnion kuetzingianum, Mougeotia* cf. *depressa, Mougeotia* cf. *laevis* and *Zygnema* cf. *cylindrospermum* were all present in at least one of the severely affected sites sampled. *K.*

acidophilum Novis was also present in 80% of the severely affected sites and was often dominant, e.g. it was the only species at Mine Drainage Causeway where if formed prolific filamentous growths. *Euglena mutabilis* formed extensive vivid light green mats and was present in 60% of severely impacted streams. In these it comprised on average 42% of total cell counts. The diatom flora of severely affected streams included *Frustulia vulgaris* (Thwaites) De Toni*, Navicula capitoradiata, Navicula cf. margalithi* Lange-Bertalot and the often dominant *Navicula cincta*. *N. cincta* was present in 60% of severely affected streams and constituted 23% on average of the community where it was present.

Moderately impacted sites had many of the taxa (32 taxa) from severely affected streams but also saw the inclusion of a range of different cyanophytes, *Cylindrospermum* sp. four different species of *Phormidium* and two species of *Lyngbya* among others. *Oedogonium* (narrow species) also appeared and was present at conductivities as high as 177 μ S cm^{-1} but at close to neutral pH (6.42). Ten different chlorophytes were present, now including *Ulothrix tenerrima*. Bacillariophytes increased in diversity with a total of seven taxa, yet *N. cincta* was absent, presumably because the pH of these sites was out of this organism's preferred range.

Mildly impacted sites had the highest total diversity (66 taxa), and had high estimated relative abundances and diversity of cyanophytes. Twenty-five different cyanophytes were identified. The rhodophytes *Batrachospermum atrum* in gametophyte and 'Chantransia stage' were present at many mildly impacted sites, as was a species of *Audouinella* distinguishable by hair-like extensions from the tip of filaments. *Euglena mutabilis* appeared in three sites with low pH (~3 to 4). Thirteen green algal and 21 diatom species were identified and these included many restricted to low pH habitats. Mildly impacted sites were the most numerous (n=20) and covered the widest pH range (2.96-7.53) and this is reflected in the high number of species found within this category.

Reference and marginally impacted sites also had high diversity (58 taxa). These sites also had high estimated relative abundances and diversity of cyanophytes species with twenty one different taxa identified. Both the Rhodophytes *Batrachospermum atrum* its 'Chantransia stage' and a species of *Aoudoinella* characterized by hair like terminal cell extensions were present. A range of chlorophytes were present (n=16), some abundant organisms include *Klebsormidium rivulare*, *Oedogonium* sp. *Microspora quadrata*, *Mougeotia* cf. *laevis*. Of the bacillariophytes 15 different taxa were identified, and had generally low (<5%) estimated relative abundances except for *Diatoma vulgaris* which made up ~50% of community composition at Coal Street Creek (Reefton). A vast number of species determined the relative placement of mild and reference sites within the CCA shown, the majority in low abundances.

2.4. Discussion

2.4.1. Algal diversity, dominance, and biomass

This survey supports the view that pH, conductivity, and the level of metal oxide deposition have negative impacts on benthic algae. Reduced algal diversity was observed, where total taxonomic richness within impact categories was established, and has been reported in many studies of AMD (Tate et al. 1995; Verb and Vis 2000; 2001; Sabater et al. 2003; Verb and Vis 2005). However, as in this study, it has been noted that numerous taxa are tolerant of conditions within AMD waterways even at the most highly acidic sites (DeNicola 2000; Sabater et al. 2003). For this reason among others, reduced diversity according to AMD impact is not clear where biotic indices are used alone. Biotic indices are accepted to fail as useful indicators of stress in certain circumstances, even where distinct trends are happening (Hill et al. 2000a and references therein), which is the case here. Simpson's diversity index, which takes into account species richness and evenness, and the Berger-Parker index of dominance, are not statistically significantly different between impact categories. However, they do indicate that severely impacted sites have higher dominance (the ratio of the most abundant taxa to all others) and lower diversity. AMD communities are often dominated by a small number of taxa (McKnight and Feder 1984; Tate et al. 1995; Verb and Vis 2000; 2001). This was also indicated by the Berger-Parker index and was clear at certain AMD sites, for example Mine Drainage Causeway was 100% dominated by *Klebsormidium acidophilum* where it was forming prolific growths.

Algal cover estimates and the estimate of algal biomass were significantly greater in the severely impacted, low pH streams sampled, which is in accordance with some studies (Muller 1980; Mulholland et al. 1986; Verb and Vis 2001; Sabater et al. 2003) but not others (Kinross et al. 1993; Anthony 1999; Hill et al. 2000a; Verb and Vis 2005). Verb and Vis (2005) found a loose inverse relationship between biomass and community dominance of diatom and macroalgal communities in AMD. They stated that where diatoms dominate low biomass may be expected, but where macroalgae dominate high biomass may be expected at severely impacted sites. They however did not speculate as to mechanisms driving macroalgal vs. diatom dominance within AMD streams, although it may be an issue of guild competition or zonation (e.g. Passy 2007). Niyogi et al. (1999; 2002) have however found that increases in algal biomass are limited by the rate of precipitate deposition, which varies according to pH and metal ion concentrations (Younger et al. 2002). The type of metal oxide has also been found to play an important role, and iron oxides the predominant type deposited in AMD streams in New Zealand may be less harmful than aluminium oxides (Niyogi et al. 1999). The findings of Niyogi et al. (1999; 2002) agree with those of this study, where precipitate deposition was estimated to be at its highest, algal cover and biomass is very low or is absent (e.g. Coast Road Creek). High rates of deposition may smother algae inhibiting photosynthesis or where the entire substrate is covered in the silty material, algal propagules may have difficulty colonizing these surfaces (Anthony 1999). It may be that colonization is not the problem, and filaments may begin growing but are removed where attachment on a finely silted substrate is overcome by drag imposed by the surrounding water. Certain species are adept at growth on these surfaces, where they are directly associated with the benthos and remain within the boundary layer (e.g. *N. cincta, E. mutabilis*), or certain filamentous species may be more adept at forming positive attachment in a silty substrate (e.g. *K. acidophilum*, *M. kuetzingianum*).

As outlined earlier there are several reasons that may explain high biomass in many severely affected streams: tolerant species are stimulated where physicochemical conditions are optimized for that particular species e.g. pH (Novis 2006; Von Dach 1943); competitors may senesce due to altered physicochemical conditions, releasing tolerant species from interspecific competition (Niyogi et al. *In press*); elevated metal concentrations may themselves act as a subsidy (Niyogi et al. 1999); or where physicochemical conditions exclude grazers (functionally or otherwise) thus releasing algae from any top-down control that would otherwise be occurring (Rosemond et al. 1993; Graham et al. 1996; Niyogi et al. 1999). Other factors such as flow rate, physical disturbance and nutrient flux may also be critical for determining biomass accrual and increased productivity in low pH habitats (Biggs and Gerbeaux 1993l; Biggs and Smith 2002). Here significant differences in biomass were due primarily to the prolific growths of *K. acidophilum* and were common in severely affected, stable AMD habitats, where precipitate deposition was not occurring at high rates.

2.4.2. Phyla differences across a gradient of AMD stress

As is the case here, many AMD studies suggest that chlorophytes dominate the macroalgal flora (Verb and Vis 2001; 2005). Chlorophytes had the highest estimated relative abundance in all categories, although marginally so in reference and mild. This is characteristic of New Zealand periphyton (Biggs, 1990) and characteristic of many periphyton communities in streams worldwide (Sheath and Burkholder 1985). Also as in other studies, the degree of impact affects representation of different phyla, which is not unexpected given the severity of the gradient sampled (Verb and Vis 2005).

2.4.3. Algal communities and the mechanisms driving structure

The most significant mechanism influencing community structure across the gradient sampled was pH. It had the highest correlation with the species matrix in every CCA analysis carried out and had significant relationships, positive and negative on the abundance of a number of individual taxa. That pH was the primary determinant of community composition helps to explain why the impact categories had indiscrete (conductivity based) groupings in the total community CCA analysis of this study. The dominant influence of pH across the gradient sampled becomes clear where pH categories are used in the same analysis (Appendix 3). Verb and Vis (2000; 20001; 2005) also found, across each of their studies, that pH was the most influential variable affecting community composition. In two of their studies these authors also found distinct differences between AMD sites and sites they refer to as reclaimed and non-impacted sites. CCA biplots grouped their sites in discrete clusters according to these categories (Verb and Vis 2000; 2001). In these earlier studies however, they did not sample along such a broad AMD gradient as this study, they stated in their 2001 paper, using the same sites in their diatom paper (Verb and Vis 2000), that most of their reclaimed and non-impacted sites had pH vales of 6.6-8.2, while their AMD sites ranged in pH from 2.6-3.3. In an experimental study Tease and Coler (1984) also found that pH had the most severe impact on periphyton communities, far out weighing the effects of dissolved aluminium, other heavy metals and even hydrocarbons.

Dissolved metals are also known to be toxic to algae (Soldo and Behra 2000; Guasch et al. 2004) and influence their distributions within a watershed (Hill et al. 2000a). In AMD streams conductivities are indicative of metal ion concentrations (Younger et al. 2002) and in this study conductivity was also found to be highly correlated with the species matrix in each CCA carried out. Precipitate deposition was another AMD variable that was important in these analyses helping to explain the distribution of sites based on their species composition.

Microthamnion kuetzingianum is one organism known to have positive associations with iron hydroxide precipitates deposited on the stream benthos (John et al.2002), in contrast to the negative associations it is known to have (McKnight and Feder 1984; Niyogi et al. 1999).

A number of other variables were significantly correlated with the species matrices and are known to be important factors influencing benthic algal communities, depth, geography, month, temperature, geographic location (Sheath and Burkholder 1985; Biggs 1990; Peterson 2007). Temperature however is correlated with both pH and conductivity making it hard to ascertain the strength of this variable.

Macroalgae respond in a very similar way to the entire community (Appendix 2) because they were the dominant organisms sampled, making up 71% of the collective species matrix. Diatoms made up 10% and euglenophytes and other unicellular microscopic algae made up the remainder. Macroalgae were dominant, as this is characteristic of New Zealand periphyton (Biggs 1990) and AMD algae (Sabater et al. 2003) and the sampling protocol was not aimed at diatoms (see methods). While the macroalgal and entire species matrices are more robust in terms of the amount of data collected, the diatom matrix does better separate out sites based on the level of impact. The diatom CCA also suggests that geography and month have a stronger influence on this component of the community.

As is the case with many field surveys that have interacting stressors, the individual effects on a community are very difficult to decouple without experimentation (Tease and Coler 1984; Hirst et al. 2004). As may be expected the hydrogen ion activity (Tease and Coler 1984; Hirst et al. 2004), conductivity which in AMD is indicative of dissolved metal concentrations (Younger et al. 2002; Hill et al 2000a; Hirst et al. 2004), and the deposition of metal oxides (Niyogi et al. 1999) influence community composition individually and collectively. Unfortunately as other authors have found the effects of heavy metal contamination (conductivity) and pH are hard to disentangle (Verb and Vis 2005). However, where individual organism correlations are taken into account and where pH categories instead of the conductivity dominated cluster categories are used to visualize the data set, it becomes clear that pH is a much stronger driver of change across this AMD gradient (Appendix 3).

The survey indicates that a characteristic community often develops in severely affected mine drainage streams. Algal cover and biomass increase as AMD impacts increase, although this is dependant upon the rate of metal oxide deposition and physical disturbance among other factors. This community is similar in species composition to AMD communities elsewhere. Also, diatoms may be the best assessors of AMD impact. The deposition of iron oxides may have the most significant impacts on algal communities tolerant of severe AMD, and was most influential in determining biomass. The greatest factor influencing community structure across the AMD gradient is pH, but is closely followed by conductivity. Also important are temperature (although it is closely correlated with all AMD stress variables), depth, the month of sampling and geographic location.

Chapter 3: Temporal variation in periphyton across an AMD gradient

3.1. Introduction

Space and time interact to shape lotic communities, on a continuum from short-term local scales, to evolutionary-global scales (Minshall 1988). Temporal change affects both the taxonomic composition (Sheath and Burkholder 1985; Oemke and Burton 1986) and biomass (Biggs and Close 1989; Sherwood and Sheath 1999; Hayward 2003) of stream periphyton. This change is often due to seasonal changes in climate, where season and climate cause a range of physicochemical factors vary through time (Cummins et al. 1984; Sheath and Burkholder 1985). Disturbance is possibly the most important broad-scale factor of stream ecosystems and is controlled by the largely stochastic and temporal nature of climate (Resh et al. 1988). The time since last disturbance and disturbance frequency are known to structure periphyton composition (Biggs and Smith 2002), and this may be especially pertinent when considering communities of disturbed West Coast streams (Winterbourn 1981). Temporal change also occurs once disturbance has altered the structure of a benthic algal community, resetting it to an earlier successional stage (Oemke and Burton 1986) in which the processes of immigration and succession proceed (Eddy 1925; Oemke and Burton 1986; Stevenson et al. 1991; Mosisch and Bunn 1997). Where floods are frequent, the development of a late successional community will generally not occur and biomass will remain low (Biggs and Close 1989). A range of other habitat variables important to periphyton vary according to season and include: light, water temperature, water velocity, water depth and nutrient flux (Sheath and Burkholder 1985; Biggs and Price 1987; Biggs and Close 1989; Biggs and Gerbeaux 1993; Mosisch and Bunn 1997). Seasonal changes in the above habitat variables are in some instances known to be as important in structuring periphyton taxonomic composition as the far more obvious effects of flood events (Oemke and Burton 1986).

In order for an ecological survey to be rigorous it is necessary to attempt to account for sources of variation that may influence community structure, such as temporal variation, so as not to confuse this change with change induced by an anthropogenic stressor (Vaultonburg and Pederson 1994; Clements and Kiffney 1995). The aims of this part of the study are: 1) to determine the relative extent of temporal change within AMD algal assemblages; 2) to determine the relative extent of temporal change within non-AMD reference algal assemblages; and 3) to compare the extent of change between community types.

3.2. Methods

3.2.1 Survey methods

This survey was carried out at seven sites directly surrounding Reefton, South Island, New Zealand. Each site was visited three times. Due to the high frequency of spates that occurred on the West Coast while conducting this survey and the known responses of communities to hydraulic disturbance (Biggs 1996), sites were not surveyed until communities had a maximal chance to respond (Biggs and Kilroy 2000), although the suggested period of 4 weeks was invariably not possible, given the levels of rain fall through out this period (Figure 3.2). Therefore, collection dates varied and spanned from April 2006 until February 2007. Sites were assigned to the impact categories of the initial survey according to the physicochemical ranges of these categories. The sites were composed of two reference, one mildly, three moderately and one severely AMD impacted site. The survey methods otherwise followed those of the general survey described in Chapter 2.

3.2.2. Study sites

Seven sites were chosen from the surroundings of Reefton, on the basis of similarity of size, flow, depth, width, temperature and canopy cover. They encompassed a range of AMD impact. The vegetation at Burkes reference Creek was dominated by Gorse (*Ulex europeas*). At all others the vegetation was generally similar and was a Beech mix including Mountain Beech (*Nothofagus solandri*) and Red Beech (*Nothofagus fusca*), with a variable understorey that was generally a fern/broad leaf mix.

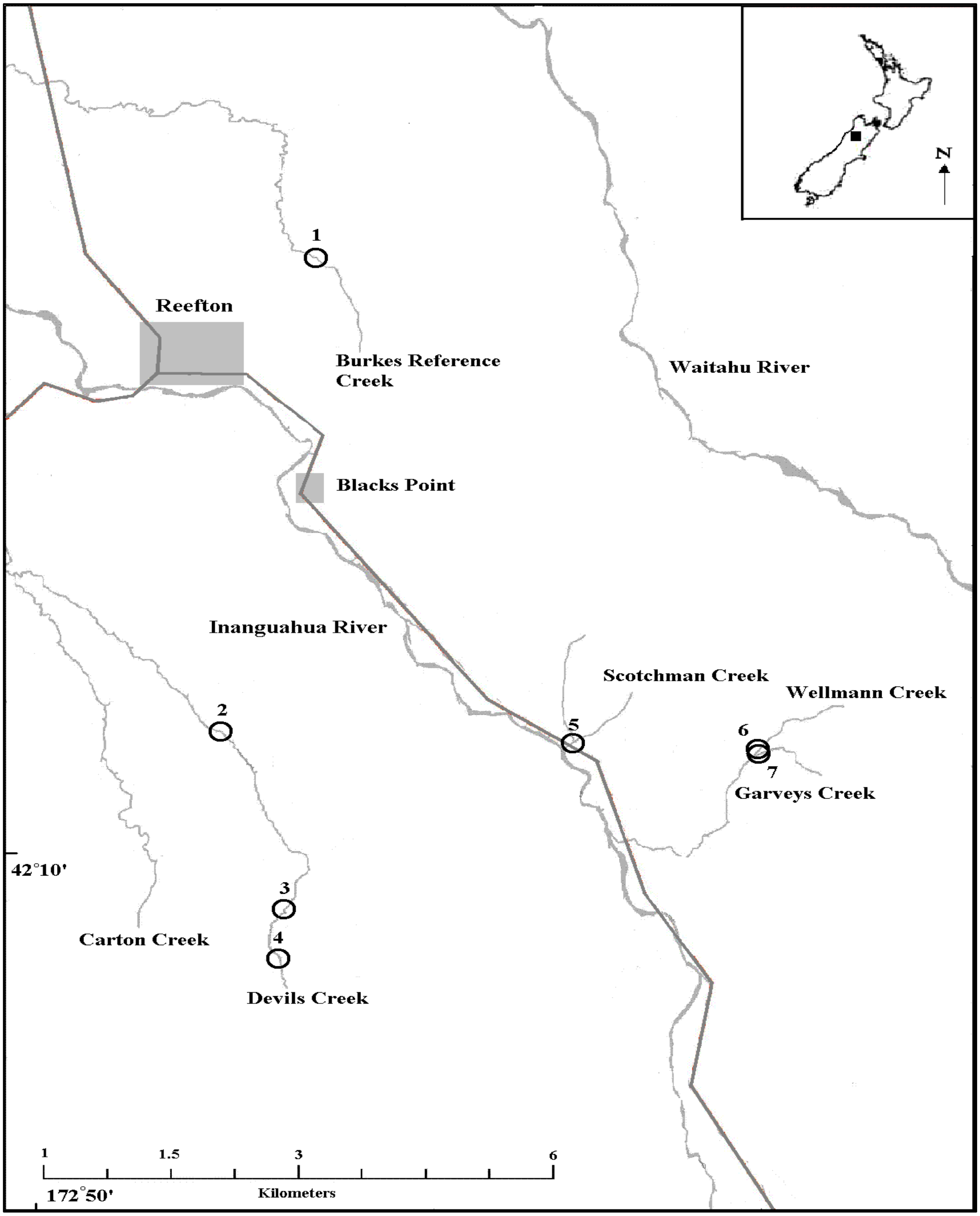

Figure 3.1. Locations of the 7 sites surveyed to assess temporal variation in periphyton communities from April 2006 to January 2007 (ESRI 2006). The sites were: 1) Burkes Creek, 2) Progress junction, 3) Globe gate, 4) Alborn car park, 5) Scotchman Creek, 6) Wellman Creek and 7) Garveys Creek.

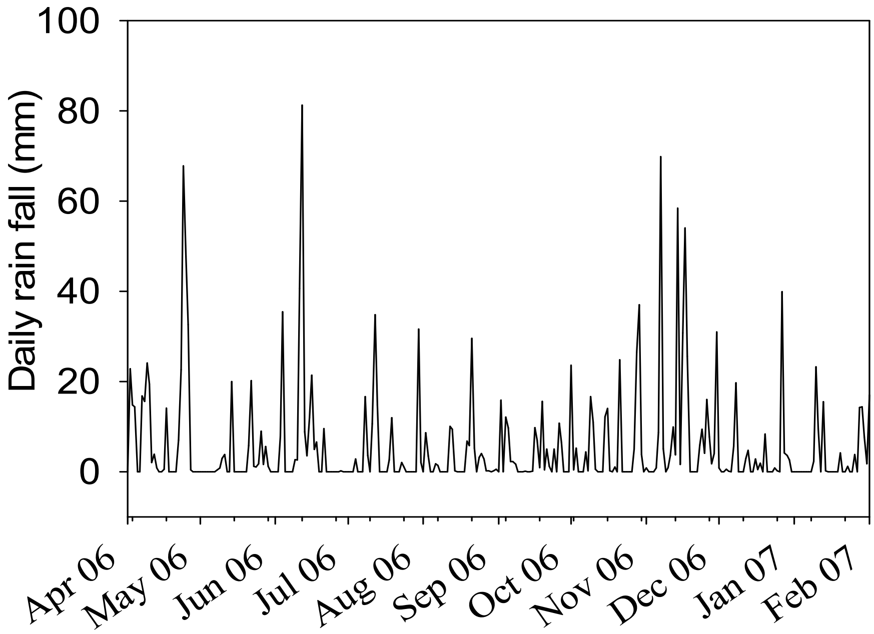

Figure 3.2. Daily rain fall from April 2006 to March 2007, data courtesy of NIWA, Site 211802 Reefton EWS.

Table 3.1. Site summary statistics of key water chemistry and physical variables between the seven temporal sites (mean values shown): pH, conductivity (cond.), the precipitate index (PI), surface water velocity (SV), stability (pfankuch), and substrate index (SI).

Site	Month sampled	Degree of impact	pH	Cond. (μ S cm^{-1})	PI	T (ºC)	SV (m/s)	Stability	SI
Burkes reference	Sep, Nov, Feb	Reference	7.1	41.7	0.3	11.4	0.9	Good	5.9
Scotchman Creek	Sep, Nov, Feb	Reference	7.0	41.1	0.3	11.1	0.4	Good	5.8
Progress Junction	Apr, Jan, Feb	Mild	7.0	95.7	1.7	14.1	0.4	Good	6.1
Alborn car park	Apr, Jan, Feb	Moderate	4.7	168.3	4.0	10.8	0.1	Good	5.2
Globe gate	Apr, Jan, Feb	Moderate	5.6	129.4	2.0	10.7	0.2	Good	5.9
Wellman Creek	Sep, Nov, Feb	Moderate	4.6	274.0	3.7	13.4	0.8	Good	5.3
Garveys Creek	Sep, Nov, Feb	Severe	3.6	548.0	3.7	14.2	0.7	Good	5.2

3.2.3. Data analysis

A two-way ANOVA, to explore relationships between community metrics, level of impact and time could not be used due to an unbalanced design, so a series of single factor ANOVAs had to be used. The ANOVA's tested differences between physicochemical conditions and month of sampling, and between community metrics and month of sampling. Normality was tested and Tukey's HSD tests were employed to establish significance between factor levels within significant tests (StatSoft Inc. 2006).

A CCA analysis was also run to identify relationships between the community matrix and the environmental matrix (McCune and Mefford 1999).

3.3. Results

3.3.1. Temporal change in stream physicochemical characteristics.

At AMD impacted sites, each of the variables indicative of this impact (pH, conductivity and precipitates) varied through time across the gradient of impact sampled (Fig. 3.3; Table 3.1). The severely impacted Garveys Creek exhibited mild changes in pH (3.3-3.9) but more marked changes in conductivity (400-820 μ S cm^{-1}). The level of precipitates changed markedly also, with an estimate of 2 during November (2006) compared to September (2006) which had the maximum possible estimate of 5. The moderately impacted sites also had some variation in pH, but greater changes were occurring in conductivity. Marked changes in the level of iron oxide precipitates were also occurring in these streams, particularly Wellman and Devils Creek at Alborn Start, where both sites received the maximum estimate of 5 and a minimum estimate of 2.

Across all sites, water temperature and surface water velocity differed significantly over time (Table 3.2.). Water temperature was significantly higher in February than in April (Tukeys HSD, P=0.0027), September (Tukeys HSD, P=0.006) and November (Tukeys HSD, P=0.023). Surface water velocity was significantly higher during November than January (Tukeys HSD, P=0.021) and February (Tukeys HSD, P=0.003). September had higher, but not significantly so, average surface water velocities than January (Tukeys HSD, P=0.26), February (Tukeys HSD, P=0.082) and April (Tukeys HSD, P=0.57) which were very similar.

Table 3.2. Summary statistics from a series of single factor ANOVAs testing differences between selected physicochemical variables and sampling months (mean values shown with ± SE, significance at P<0.05).

	January (n=3)	February (n=7)	April (n=3)	September (n=4)	November (n=4)	F	P
Temperature	12.0 (1.12)	15.4 (0.73)	9.27 (1.12)	10.3 (0.97)	11.15 (95)	$7.63_{(df=4)}$	0.001
Surface velocity	0.24 (0.18)	0.20 (0.12)	0.38 (0.18)	0.73 (0.15)	1.1 (0.15)	$6.31_{(df=4)}$	0.003
Pfankuch	54.3 (5.64)	54.9 (3.7)	69 (5.6)	61.3 (4.9)	67.3 (4.9)	$1.9_{(df=4)}$	NS
Depth	0.14 (1.4)	0.45 (0.9)	0.12 (1.4)	2.8 (1.2)	0.47 (1.2)	$0.88_{(df=4)}$	NS

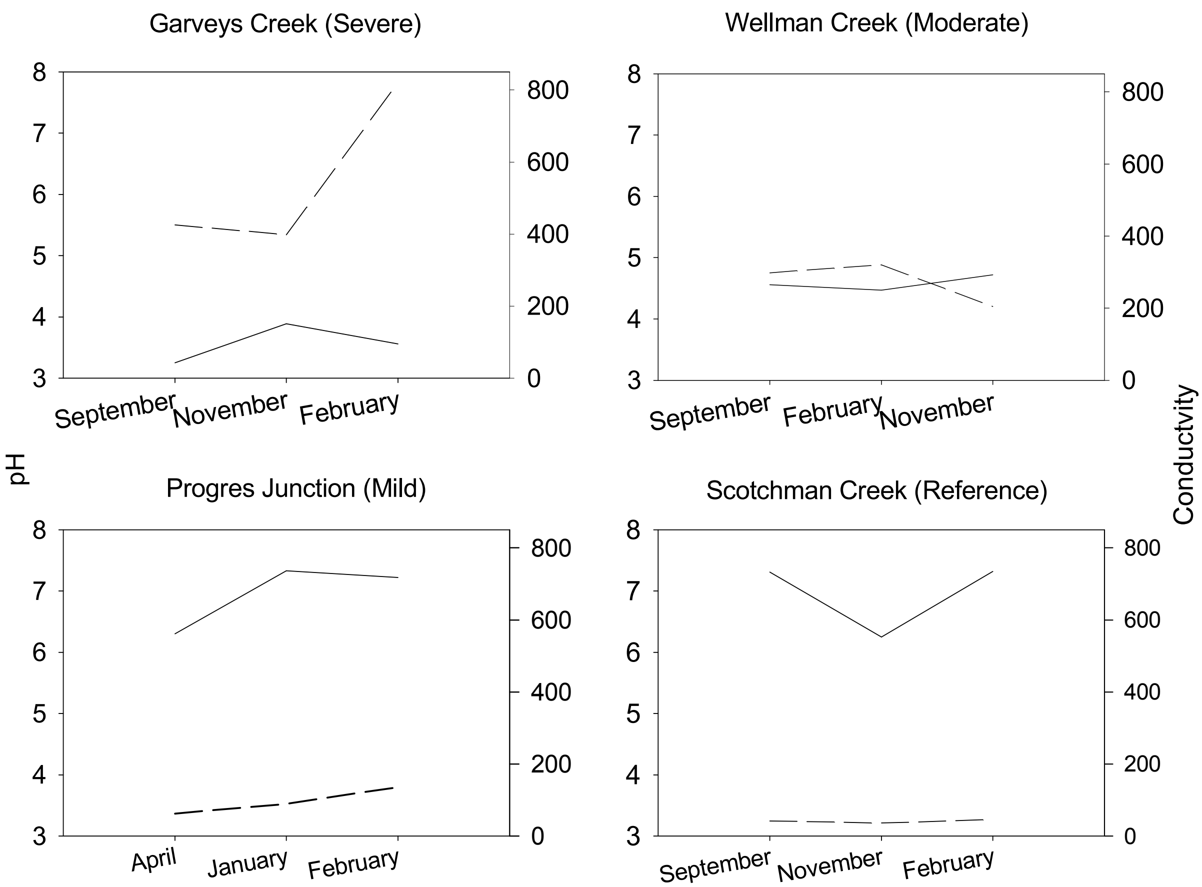

Figure 3.3. Contrasting conductivity (dashed line, right hand Y axis) and pH (solid line, left hand Y axis) measurements through time at select sites of varied impact.

3.3.2. Comparing periphyton communities across an AMD/Time gradient

Taxonomic richness varied by month at each site sampled, across all levels of impact sampled (Fig. 3.4). Within reference sites, algal cover, biomass, richness, Simpson's Index and the Berger-Parker index of dominance did not significantly differ with month of sampling. Within sites impacted by AMD, algal cover, biomass, Simpson's Index and the Berger-Parker index of dominance did not significantly differ with month. Richness differed significantly in AMD impacted sites (ANOVA $F=4.55_{df=4}$, P=0.024), where April had significantly higher taxonomic richness than February (Tukeys HSD test P=0.027). During January the Alborn car park site had extremely high estimated biomass and algal cover (~100%), which was primarily *Microspora quadrata* Hazen (66%) and *Klebsormidium acidophilum* Novis (20%). This site had the lowest recorded surface water velocity (0.07 m S^{-1}) during this study component.

Temporal changes in relative abundances of phyla within sites show varying trends (Fig. 3.5; 3.6). Both Garveys Creek (severe) and Wellman Creek (moderate) were consistently completely dominated by chlorophytes (100%).

Both of the reference sites showed marked changes in the representation of phyla. During September, Scotchman Creek had high estimated relative abundance of cyanophytes (90%) while rhodophytes and bacillariophytes were also present (Fig. 3.5). By November bacillariophytes (53%) and cyanophytes (45%) were most abundant. During February rhodophytes (60%) and bacillariophytes (29%) were most abundant while cyanophytes and chlorophytes were also represented.

The community at Burkes Creek shows marked changes from February, where composition consisted of cyanophytes (56%) and bacillariophytes (33%), and by November and February chlorophytes dominated (~90%).

Composition changes down Devils Creek were also occurred (Fig. 3.6). During April, February and January, both the Alborn car park and the Globe gate site were dominated by chlorophytes (100%), with the exception of the Globe gate site during February, in which only three diatom frustules were observed. This low abundance was due to very recent road workings at this site. The Devils Creek Progress Junction site had a variable flora. During April rhodophytes were dominant (85%), which shifted to bacillariophytes in February (100%) and chlorophytes (99%) in January.

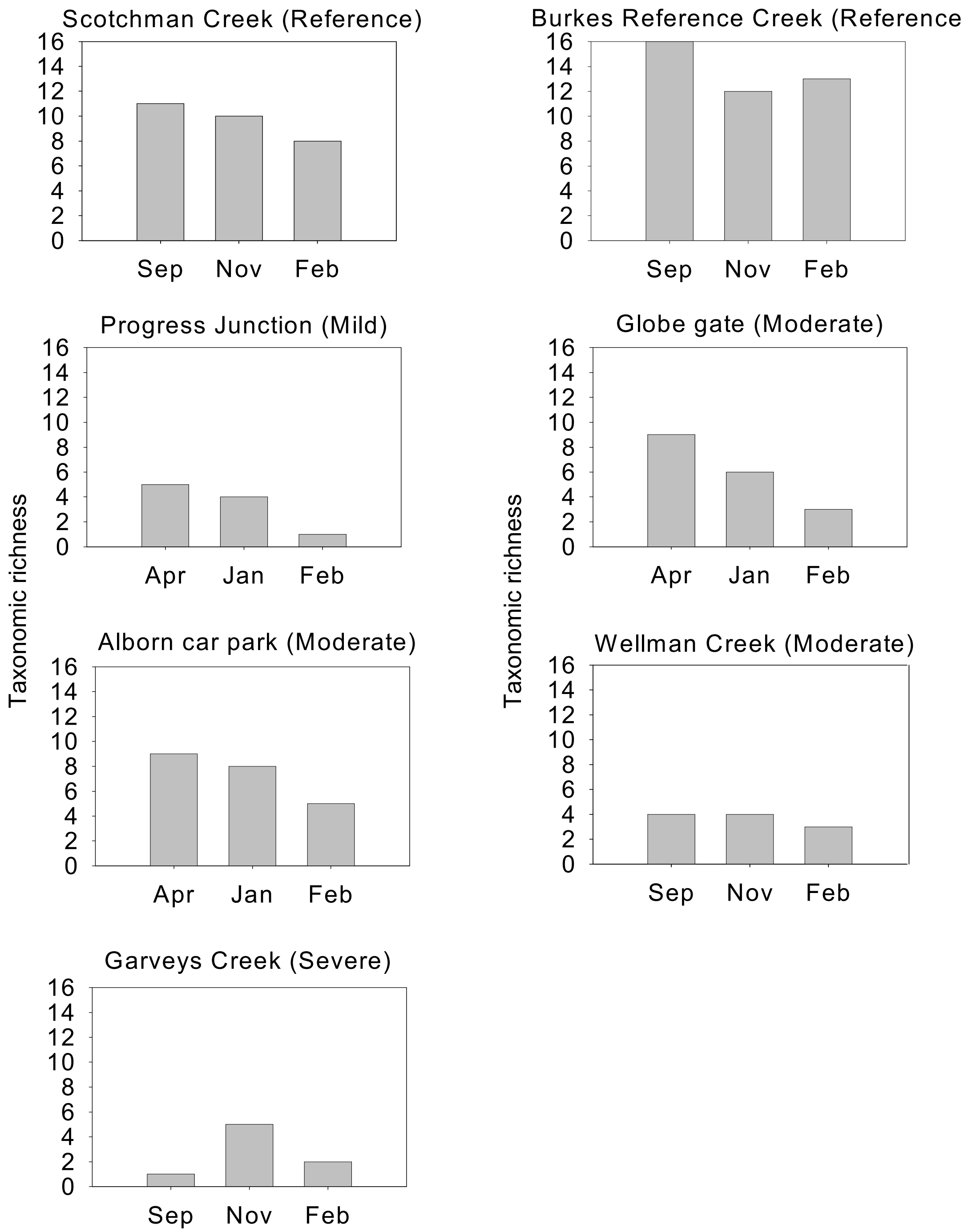

Figure 3.4. Taxonomic richness at each site by month of sampling (April 2006 – February 2007).

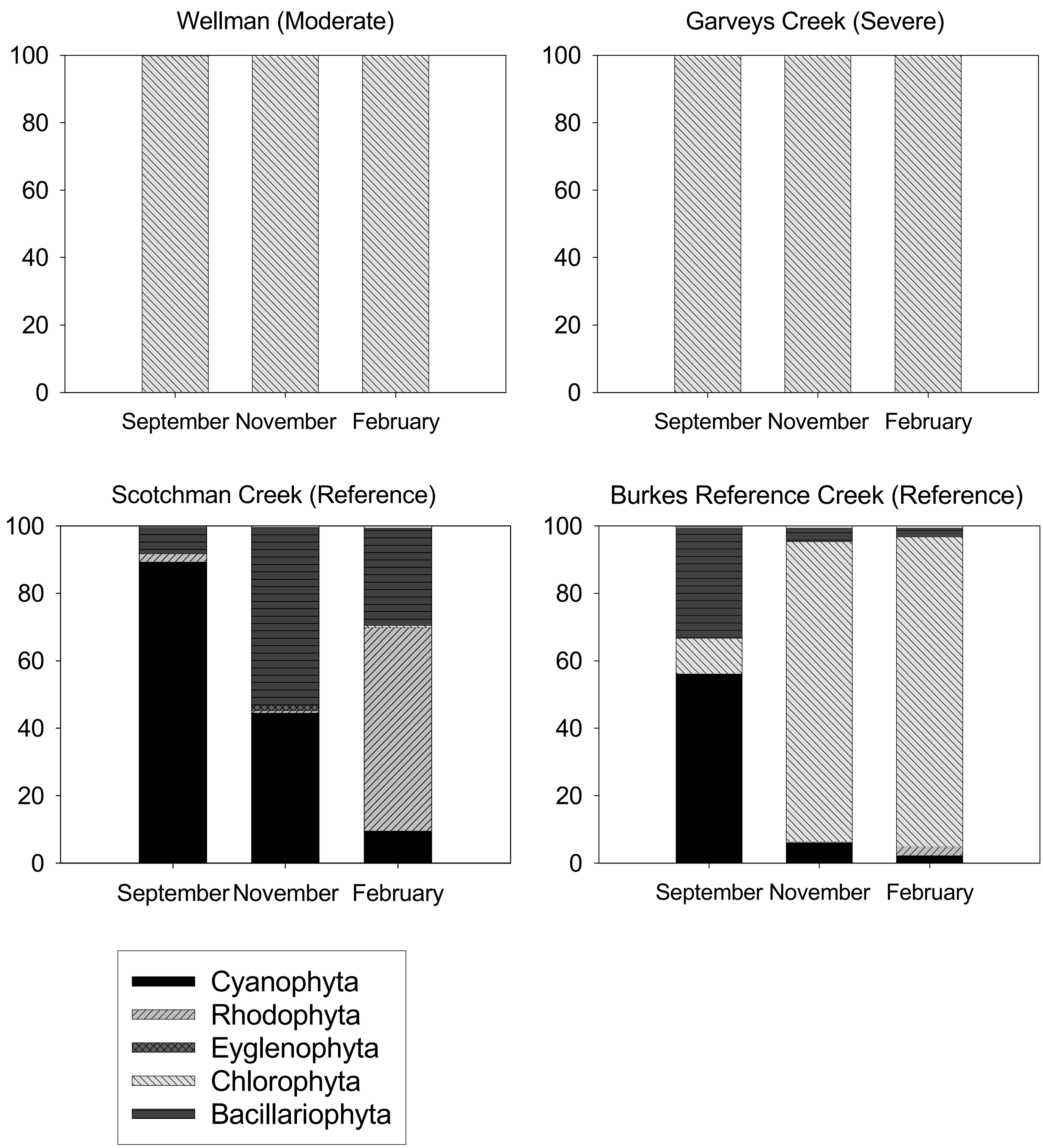

Figure 3.5. Community composition, determined using estimated relative abundances, by month of sampling (April 2006 – February 2007) at Wellman Creek, Garveys Creek, Scotchman Creek and Burkes Reference Creek.

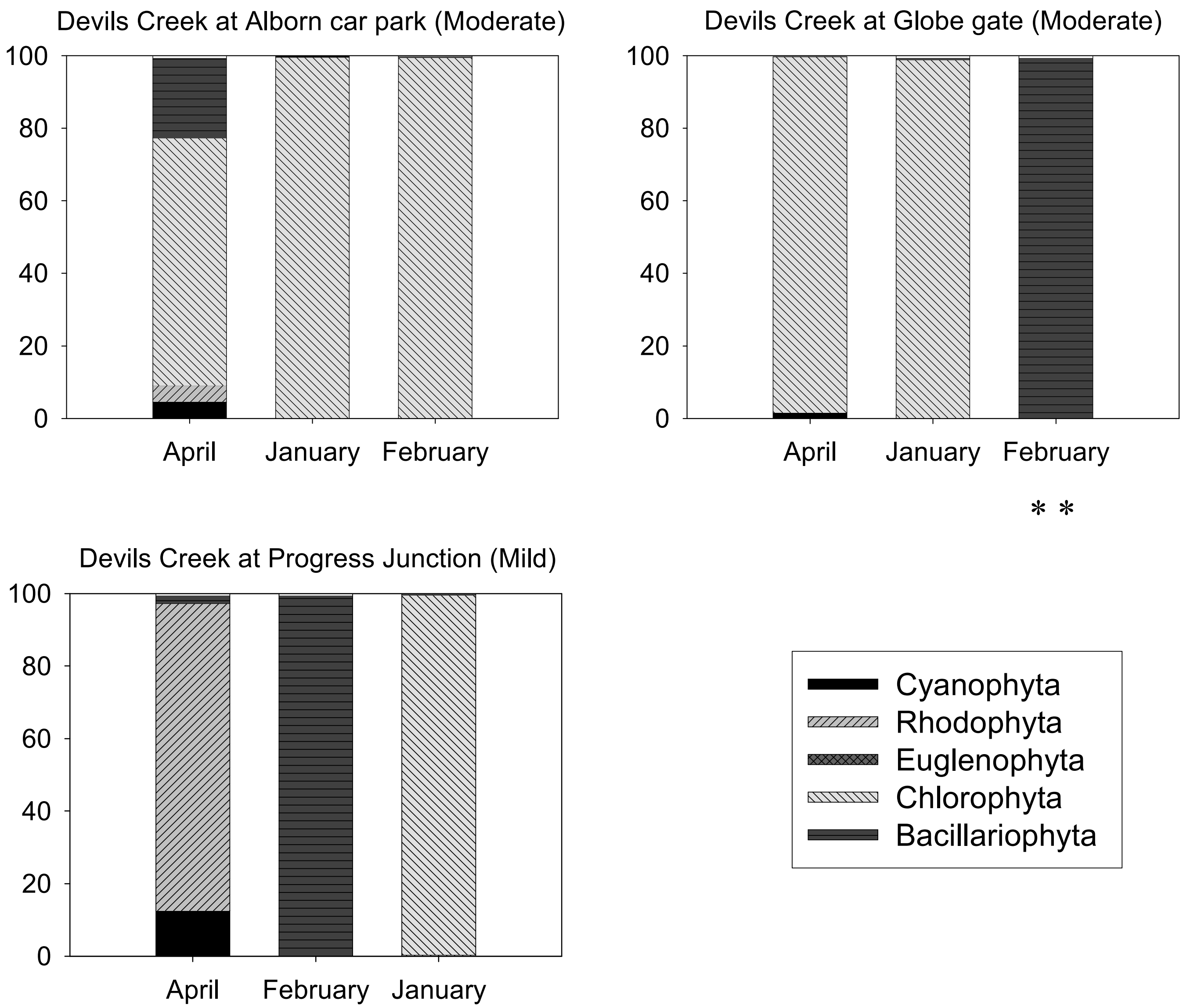

Figure 3.6. Community composition, determined using estimated relative abundances, by the month of sampling (April 2006 – February 2007) at sites down Devils Creek, Devils Creek at Alborn car park, Globe gate and Progress Junction. * * Indicating the site and month where recent road workings appeared to influence community composition.

A total of 58 taxa were identified from the 7 sites and many occurred in low abundances and frequency across sites. Importantly several taxa were noted only once, but in significant levels within the assemblage. These taxa include *Cyanodermatium* sp. and *Nitzschia clausi,* both in Scotchman Creek during November (2006), occupying 10% and 15% of community composition respectively, while *Phormidium inundatum* (Kützing) Gomont made up ~15% of this sites composition in September (2006). *Bulbochaete* sp. made up ~20% of the community at Devils Creek Globe Mine site during April (2006).

Where dominant taxa were observed (i.e. defined in this instance as those that occurred in at least two different months or two different sites), some distinct temporal patterns of occurrence (Table 3.3) and relative abundance with regard to level of impact were noted. Few taxa (5) were identified within the severely impacted Garveys Creek site. *Gloeocystis* sp. had high abundances during September (2006) and November (2006) where it made up 100% and 86% of community composition respectively. During November (2006), Garveys Creek also had *K. acidophilum, Zygnema* cf. *cylindrospermum*, *M. quadrata* and *Microthamnion kuetzingianum* in low abundance. During February (2007), the previously abundant *Gloeocystis* sp. was absent and composition was 99% *M. kuetzingianum* and <1% *K. acidophilum*.

The three moderately impacted streams had a much wider range of taxa (22), many of which had patchy distributions over time. *K. acidophilum, M. quadrata, Z.* cf. *cylindrospermum* and *M. kuetzingianum* were all dominant within moderately impacted streams, having high frequency across sites and abundances within sites. *K. acidophilum* was in 7 of the 9 sites and made up an average of 22% of community composition of these sites. *K. acidophilum* was absent from both of the Alborn and Globe Mine Devils Creek sites during February. *M. quadrata* was often abundant comprising up to 90% of community composition and also exhibited seasonal variation in abundance. *M. kuetzingianum* was frequent but had consistently low abundance (~10%) and exhibited little seasonal variation. *Z.* cf. *cylindrospermum* was present only at Wellman Creek during September and November, but then made up on average ~55% of assemblage composition.

The mildly AMD impacted Progress Junction site exhibited marked seasonal variation in community composition and across all months and had moderately high numbers of observed taxa (9), but was very temporally variable. During April (2006) this site was dominated by *Audouinella* sp. (85%), during January (2007) *Draparnaldia mutabilis* dominated (99%) and during February (2007) *Navicula cryptocephala* Kütz. was the only alga found.

The two reference sites were consistently the most taxonomically rich and across all sites and months had high numbers of observed taxa (40). Taxa present rarely dominated community composition, and these sites also exhibited the most seasonal variation. Both reference sites differed from each other and differed markedly between months sampled.

The first CCA axis correlates well with the AMD contamination variables such as pH, conductivity and precipitates. While the second axis correlates well with two non-AMD contamination variables; month and canopy cover (Fig. 3.4). The third axis (not plotted) was correlated with month and highly so with the stream stability index (Pfankuch 1975). These axes combined explain 23.7% of the variance within the species matrix, which has strong associations with the environmental matrix (Table 3.4). A number of species were associated with sites grouped in the moderate and severe CCA bi-plot groupings (Fig. 3.3). These species include: *K. acidophilum*, *Gloeocystis* sp., *Z.* cf. *cylindrospermum*, *Mougeotia* cf. *depressa*, *M. quadrata, Euglena mutabilis, M. kuetzingianum, D. mutabilis* and *Eunotia lunaris* var. *subarcuata.* Other species were noted, but at much lower abundance and frequency within severe and moderate sites. Reference and mildly impacted sites had comparatively more variable taxonomic composition, which is indicated by their stronger divergence along Axis 2 (Fig. 3.7). These sites were more taxonomically rich and had fewer consistent species across sites and months.

Table 3.3. The presence/absence of dominant taxa by degree of impact and month of sampling, from April 2006 – February 2007.

	Severe (n=1)			Moderate (n=3)					Mild (n=1)			Reference (n=2)		
	Sep	Nov	Feb	Apr	Sep	Nov	Jan	Feb	Apr	Jan	Feb	Sep	Nov	Feb
Aphanocapsa sp.												X		
Chamaesiphon cf. *incrustans* Grunow												X	X	X
Chamaesiphon sp.												X	X	
Heteroleibleinia purpurascens (Hansgirg) Anagnostidis and Komárek												X	X	X
Lyngbya epiphytica												X		

Hieronymus														
Lyngbya aerugineo-coerulea (Kützing) Gomont												X		X
Lyngbya cf. *martensiana* (Meneghini) Gomont									X					X
Batrachospermum atrum (Hudson) Harvey				X								X		X
Batrachospermum 'chantransia stage'						X						X	X	X
Audouinella sp.									X	X			X	X
Euglena mutabilis Schmidtz				X	X		X							
Draparnaldia mutabilis (Roth) Cedergren							X	X		X				
Gloeocystis sp.	X		X			X								
Klebsormidium acidophilum Novis		X	X	X	X	X	X	X						
Microspora quadrata Hazen		X		X			X	X		X			X	X
Microspora cf. *floccosa* (Vaucher) Thuret				X										X
Microthamnion kuetzingianum Nägeli		X	X	X	X	X	X	X						X
Mougeotia cf. *depressa* (Hassal) Whittrock							X	X					X	
Mougeotia cf. *laevis* (Kützing) Archer				X									X	
Oedogonium sp.												X	X	X
Vaucheria sp.													X	X
Zygnema cf. *cylindrospermum* (West et. G.S. West) Krieger		X			X	X								
Achnanthes joursacense Hérib												X	X	X
Cocconeis placentula Ehr.													X	X
Diatoma hiemale (Roth) Heib.												X		X
Eunotia lunaris var. *subarcuata* (Naeg.) Grun.				X			X	X						
Epithemia sorex Kütz												X	X	
Frustulia rhomboides var. *crassinerva* (Brébisson) Ross.							X					X		
Gomphonema parvulum (Kütz.) Grun.												X	X	X
Navicula capitoradiata Germain												X		X
Pinnularia subcapitata Greg.							X		X					

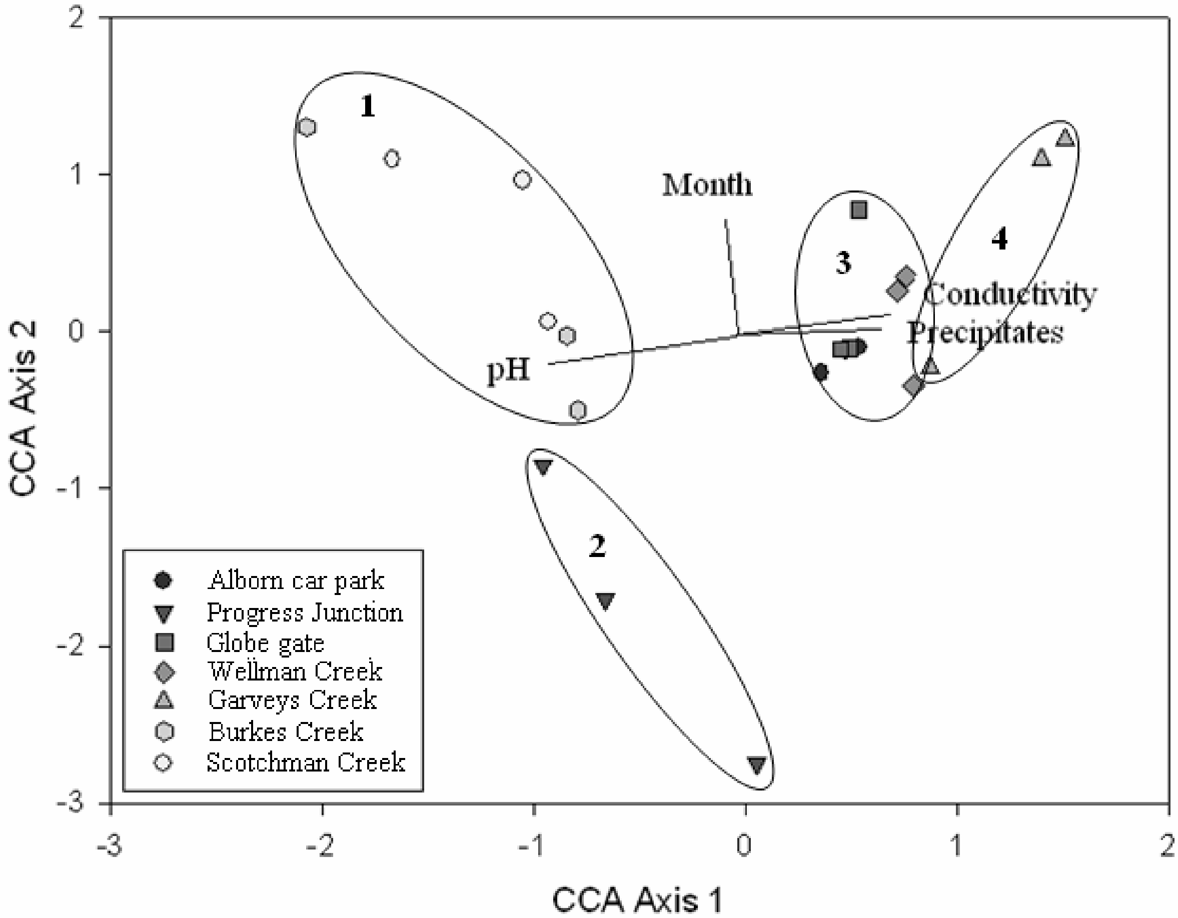

Figure 3.7. CCA biplot with the impact groupings circled, 1) Reference, 2) Mildly, 3) Moderately, 4) Severely impacted (April 2006 – February 2007).

Table 3.4. CCA summary statistics and ter Braak (1986) axis correlations with physicochemical data.

Summary statistics	Axis 1	Axis 2	Axis 3
Eigenvalue	0.93	0.84	0.71
Cumulative variance explained	8.90	17.00	23.70
Pearson correlation (Spp-Evnt)	0.99	0.96	0.94
Axis correlations (ter Braak 1986)			
pH	-0.92	-0.24	0.12

Conductivity	0.66	0.14	-0.37
Temperature	0.18	-0.34	-0.18
Precipitates	0.66	0.09	-0.11
Pfankuch	0.01	-0.34	-0.78
Surface velocity	0.04	0.15	-0.37
Canopy cover	0.16	0.40	0.23
Substrate index	-0.34	-0.08	0.03
Month	-0.08	0.60	-0.49

3.4. Discussion

Periphyton in New Zealand streams and rivers is known to exhibit seasonal patterns in standing crop and community composition, and these patterns are invariably related to abiotic factors such as disturbance, flow regime, and nutrient concentrations (Biggs and Gerbeaux 1993; Biggs and Smith 2002; Hayward 2003). Throughout the period sampled, spates were frequent and, similar to other studies carried out in New Zealand streams (Biggs and Smith 2002), disturbance appeared to be a primary driver of temporal change, although it was not expressly measured (Fig. 3.2).

Taxonomic richness has been observed to change according to season. Within this study richness did change in both reference and AMD sites, although generally not in a marked way and again appeared to be in response to disturbance (Biggs and Smith 2002) and or to fluctuations in AMD stressors (Verb and Vis 2005). Measures of diversity and evenness changed within impact categories although not significantly with regard to the month of sampling. This is interesting and shows as others have (Hill et al. 2000a) that despite the often striking changes within the community, diversity, richness and dominance are not necessarily affected.

That biomass and algal cover did not differ markedly within both AMD impacted and non-impacted streams may simply be a function of the nature of streams around the Reefton. Observations while sampling and rainfall data suggest that many of the sites sampled were highly disturbed throughout the survey, and time since last disturbance may not have been long enough for significant biomass accrual (Biggs and Price 1987). In accordance with the findings of some authors and the findings of Chapter 1, the only algal proliferation noted was in a low pH habitat, with high conductivities, that was experiencing a low, stable period of flow (Mulholland et al. 1986; Biggs and Price 1987).

Temporal change in community composition occurred at each stream and was greatest within reference and mildly impacted streams, which is comparable to the findings of Verb and Vis (2001; 2005). This may be expected where the harsh chemical and physical conditions of moderately and severely impacted streams initially constrain the number of taxa capable of growth. This results in a lower likelihood of significant taxonomic changes occurring as AMD severity increases. Communities within moderately impacted streams were dominated year round by *M. quadrata* and to a lesser extent *K. acidophilum*, which is again similar to the findings of Verb and Vis (2001), where *K. rivulare* and *M. tumidula* changed little according to season (Verb and Vis 2001).

The severely affected Garveys Creek site differed as it was dominated in September and November by a colonial green unicell (*Gloeocystis* sp.), not an algae commonly found in other AMD studies, or in the general survey of this investigation (Chapter 2). Change was noted in February when dominance changed to *M. kuetzingianum*. From personal observations however, Garveys Creek was probably the most hydraulically disturbed severe site sampled. It was noted to have changeable water chemistry with differences in pH, the level of precipitate deposited on the substrate and in particular conductivity. Importantly all of these factors are known to influence communities over short time scales (Admiraal et al. 1999; Niyogi et al. 1999; Biggs and Smith 2002; Hirst et al. 2004). Verb and Vis found pH oscillated between circumneutral and acidic in some streams and hypothesized this was occurring in others (Verb and Vis 2000; 2005). Their reasons were; two circumneutral sites were dominated by two diatoms indicative of low pH sites, and the sites had little or no buffering capacity and had low overall densities of benthic algae (Verb and Vis 2005). Reductions in pH can occur where disturbance events wash away oxidation products present on pyritic surfaces, increasing the exposure of reactive surfaces for bacterial oxidation, which in turn, may increase the concentrations of dissolved metals (Verb and Vis 2000). T-Creek was a low pH site excluded very early on from the general survey, because it had an algal flora including a range of cyanobacterial species indicative of reference streams. It was starkly out of place using ordination techniques and site groupings based on pH categories. This result was put down to incorrect physicochemical measurement or sample mixing both of which are unlikely. The most likely explanation is this stream had variable levels of AMD inputs.

Where the physicochemical conditions of acid mine drainage constrain the number of species capable of growth, fewer changes occur in community composition within affected streams along a temporal gradient. This is in contrast to reference and mild streams, where a low pH and high levels of dissolved and precipitated metals, do not have an overriding effect on the community. Where this is the case, a much broader number of taxa occur, and other factors, such as those associated with climate (e.g. hydraulic disturbance) have a far greater influence and their effects are more apparent on algal community composition and biomass (Biggs and Gerbeaux 1993; Biggs 1996; Verb and Vis 2001; Biggs and Smith 2002; Hayward 2003).

Chapter 4: Algal assemblage change down a mining impacted stream

4.1. Introduction

Periphyton communities are influenced by a wide range of environmental variables acting on both small (reach/microhabitat) and large-scales (catchment) (Biggs and Gerbeaux 1993). Over large scales, variables such as catchment geology and land use affect water conductivity and broad scale nutrient flux, influencing communities at the broadest level (Biggs 1990). Over smaller scales, a number of other variables influence periphyton, these include: light (DeNicola et al. 1992); current (Poff et al. 1990); disturbance (Biggs and Close 1989); substrate type (Tuchman and Blinn 1979; Murdock and Dodds 2007); patchiness in nutrient availability (Biggs and Smith 2002); temperature (DeNicola 1996); grazing pressure (Peterson et al. 2001) and competition (Oemke and Burton 1986, Stevenson et al. 1991). Many of these physicochemical and biotic variables change along the length of a stream, e.g. from headwaters to river mouths, thereby influencing the physical and taxonomic structure of periphyton (Vannote et al. 1980; Ward 1986; Molloy 1992).

Under natural stream conditions some authors have found no changes in longitudinal benthic algal diversity (Cushing and Rushforth 1984). Molloy (1992) found variable responses in algal diversity. She found community structure changed with current velocity and light, and included shifts in species composition of diatom guilds. A biological guild has been defined as a group of species that exploit the same class of environmental resources in a similar way (Root 1967). In this instance Molloy (1992) found diatom phenotypes best adapted to withstanding or recovering from disturbance, were most abundant in upstream disturbed sites and gave way to alternate phenotypes as stability increased. Changes in diversity may also be expected where intermediate levels of disturbance occur in mid-reach (fifth and sixth order) waterways (Vannote et al. 1980; Townsend et al. 1997) which have a broader range of potential sources of colonizers and was also observed by Molloy (1992).

This study surveys periphyton along a stream that has been impacted by AMD from a disused mine and the recent affects of opencast mining. AMD stress within a waterway should decrease down the length of a stream to which there are inflows of uncontaminated ground water, overland flows and other streams. These should dilute AMD and increase pH whilst diluting metal ions in solution (Younger et al. 2002). Ratios and concentrations of ionic metal species will change as dilution occurs and some precipitate. However, an added stress is that of metal oxide deposition (Niyogi et al. 2002).

The effects of opencast mining are different from those of AMD, and result in high levels of mineral suspensoids in solution. Of particular concern are not the coarser fractions of gravel, sand and silt, but the much smaller clay particles that have a lifetime of days or weeks within suspension (Kirk 1985). When in suspension and when trapped within the periphyton matrix, these particles interfere with light transmission and reduce primary productivity. The reduction in organic matter content of periphyton by up to 50%, reduces its quality as a food source for invertebrates (Davies-Colley et al. 1992; Quinn et al. 1992).

Where mining stress decreases with distance down stream changes in ecosystem health may be expected, reflected in the structure of algal assemblages. It must be considered that separating natural variation in community structure, such as that observed down the length of a stream, from variation associated with anthropogenic disturbance may be impossible without experimentation (Clements and Kiffney 1995; Medley and Clements 1998). Using an AMD stress gradient down a selected stream, this study will attempt to: 1) establish the relative roles of mining stressors and natural environmental factors on periphyton assemblages, and 2) ascertain whether periphyton assemblages recover to those more typical of unperturbed streams.

4.2. Methods

4.2.1. Survey methods

Ten sites were sampled down Devils Creek (Reefton). Each site, was sampled once over 4 - 5 January 2007. The survey methods follow those of the general survey (Chapter 2), although two extra environmental parameters were measured at each site. In addition to other physicochemical variables measured, TDS (total dissolved solids) were estimated using a Eutech PC 300 hand-held meter and Turbidity was measured as Nephelometric turbidity units (NTUs) using a Hach 2100P Portable Turbidimeter.

4.2.2. Study sites

Devils Creek is south of Reefton and has both AMD inputs from Alborns mine, a disused underground mine, and suspended sediment inputs from Globe opencast mine (Fig. 4.1.). Eight sites down Devils Creek and two on its tributaries, Union Creek and Oriental Creek, were sampled down a distance of 7.2 km (a 270 m vertical decrease). Vegetation surrounding sites 1 - 8, and the majority of the catchment, was dominated by *Nothofagus solandri* and *Nothofagus fusca*, with a broadleaf understorey. Sites 9 and 10 had riparian vegetation strips but were otherwise surrounded by pastureland with patches of Manuka (*Leptospermum scoparium*). Oriental Creek (site 7) was a small forested tributary of Devils Creek and at the time of sampling was markedly turbid (45.9 NTUs). The banks of Union Creek (site 5) had recently been bulldozed and an extensive area of riparian forest had been removed. This site was the drain for a recently installed settling pond for the opencast mine above this stream, where draining water was extremely turbid (112 NTUs).

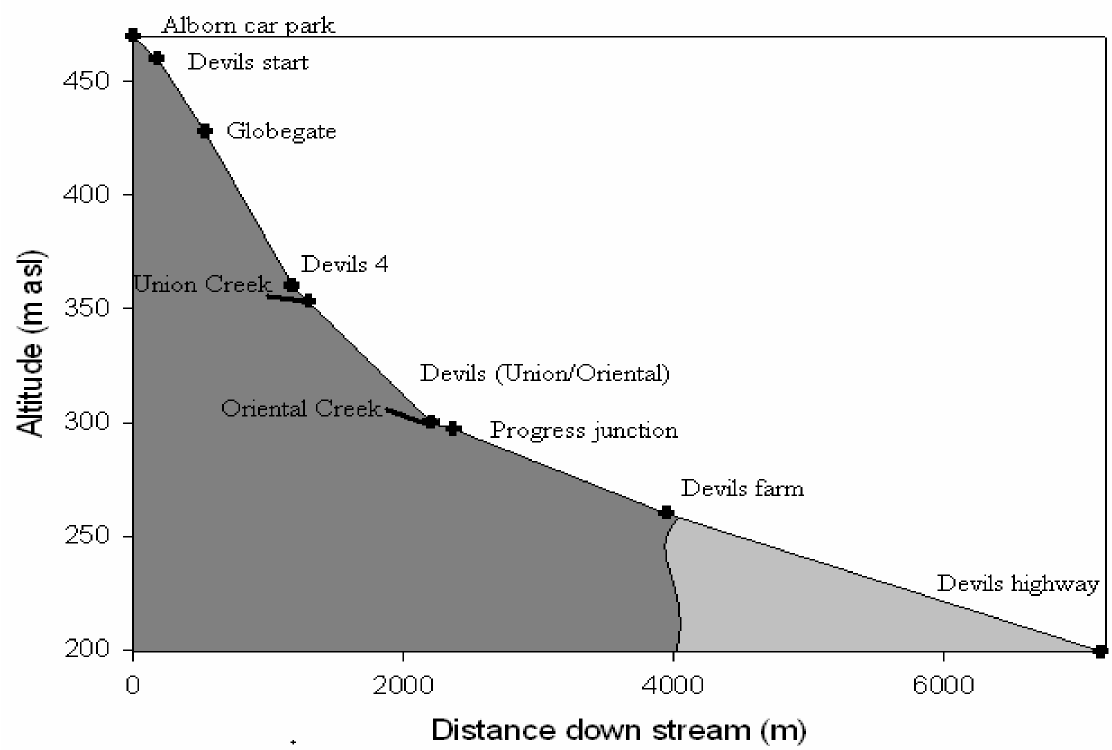

Figure 4.1. Longitudinal profile of sites sampled along Devils Creek and major tributaries. Dark grey area indicating forested catchment, light grey pastureland and Manuka. The sites are: 1) Alborn car park, 2) Devils start, 3) Globe gate, 4) Devils 4, 5) Union Creek, 6) Devils (Union/Oriental), 7) Oriental Creek, 8) Progress Junction, 9) Devils farm, 10) Devils highway (sampled 4 - 5 January 2007).

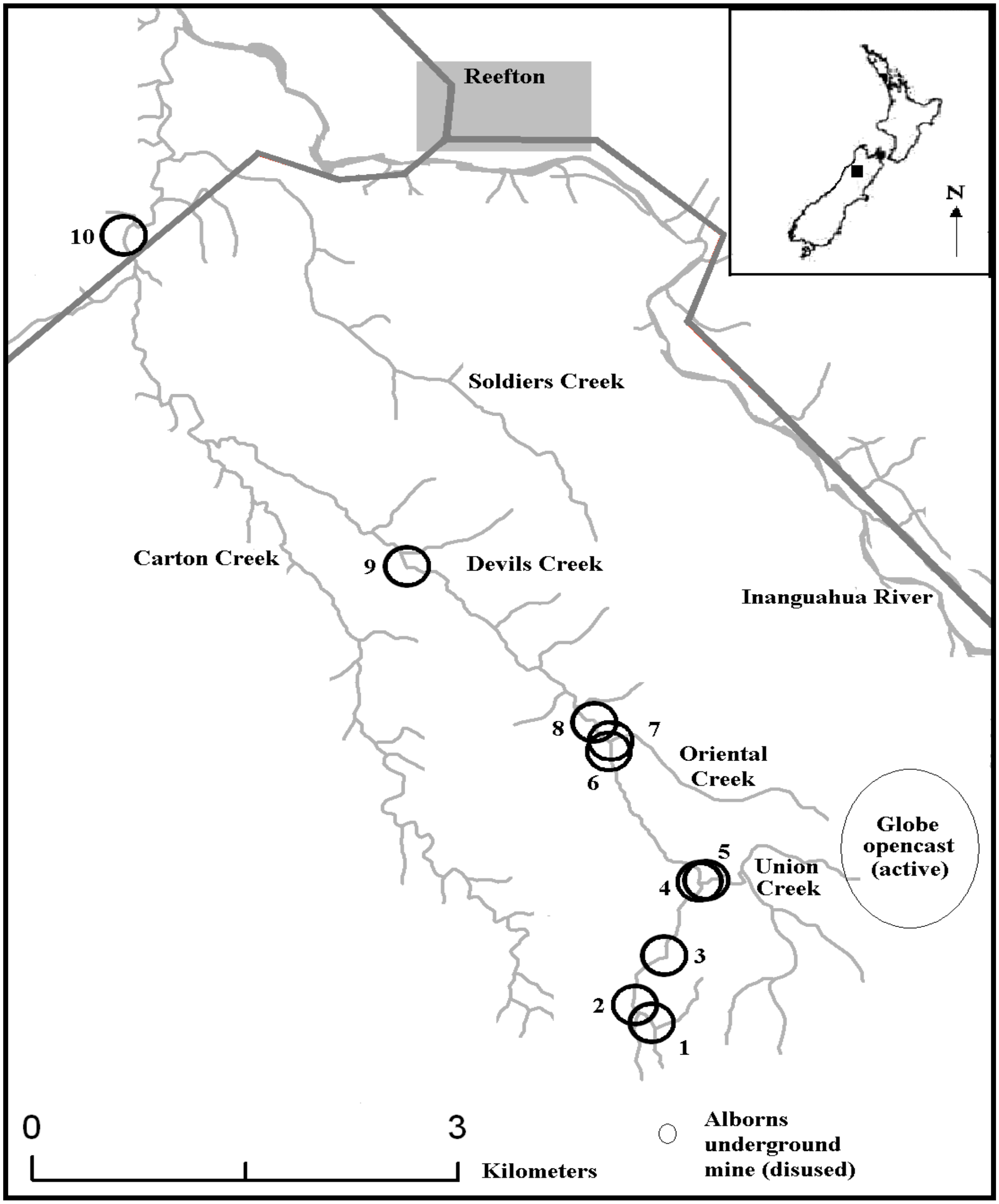

Figure 4.2. Map of the study area south of Reefton showing the study sites along Devils Creek and in two of its tributaries, Oriental Creek and Union Creek, and approximate locations of mines. The sites are: 1) Alborn car park, 2) Devils start, 3) Globe gate, 4) Devils 4, 5) Union Creek, 6) Devils (Union/Oriental), 7) Oriental Creek, 8) Progress junction, 9) Devils farm, 10) Devils highway (sampled 4 - 5 January 2007).

4.2.2. Statistical analyses

Both PC-ORD Version 4.01 (McCune and Mefford, 1999) and STATISTICA Version 7.1 (StatSoft Inc. 2006) were used for analysis of data. Canonical correspondence analyses (CCA) were run to establish which if any of the environmental variables influenced community composition, and to identify taxa strongly associated with impact groups. Several environmental variables were excluded where they were closely correlated and because the number of environmental variables cannot exceed the number of sites in a CCA analysis.

Single factor ANOVAs, Tukey's post-hoc tests and non-parametric correlation analyses were conducted to establish relationships within and between environmental variables and biological data (StatSoft Inc. 2006). Where required normality was tested using histograms, residual scatter plots and normality tests (StatSoft Inc. 2006).

4.3. Results

4.3.1. Physicochemical change

Distance from the first site and altitude were highly significantly correlated (Spearman rank, $P<0.0001$, $R = -0.99$), as such altitude was selected for all analyses and graphing purposes. Altitude (meters above sea level) was significantly (Spearman rank, $P<0.05$) correlated with pH (-0.80), conductivity (0.80), stream stability (0.70) and TDS (0.80). Temperature and surface water velocity also increased, although not significantly, with altitude. The water chemistry variables indicative of AMD severity decreased with decreasing elevation (Fig. 4.2.). Conductivity decreased relatively steadily with altitude; in contrast pH was at its lowest at the first site but quickly became circumneutral to slightly basic after the addition of suspended sediment inputs (mean 7.5). What appeared to be a brown precipitate (possibly Manganese oxide) rather than the orange $Fe(OH)_3$ was present at the highest site (site 1) and precipitates were not obvious at any other site. Turbidity was very low at the first four sites sampled, but down stream of the confluence with Union Creek (112 NTUs) was conspicuously high at 53 NTUs. After the confluence with Oriental Creek (47 NTUs) turbidity began steadily decreasing with decreasing altitude. From the first point of clay inputs (Union Creek), Devils Creek only recovered from these inputs after a distance down stream of 4.2km and a drop in altitude of 171m, where at the Devils Highway site water turbidity was 9 NTUs (ESRI 2006).

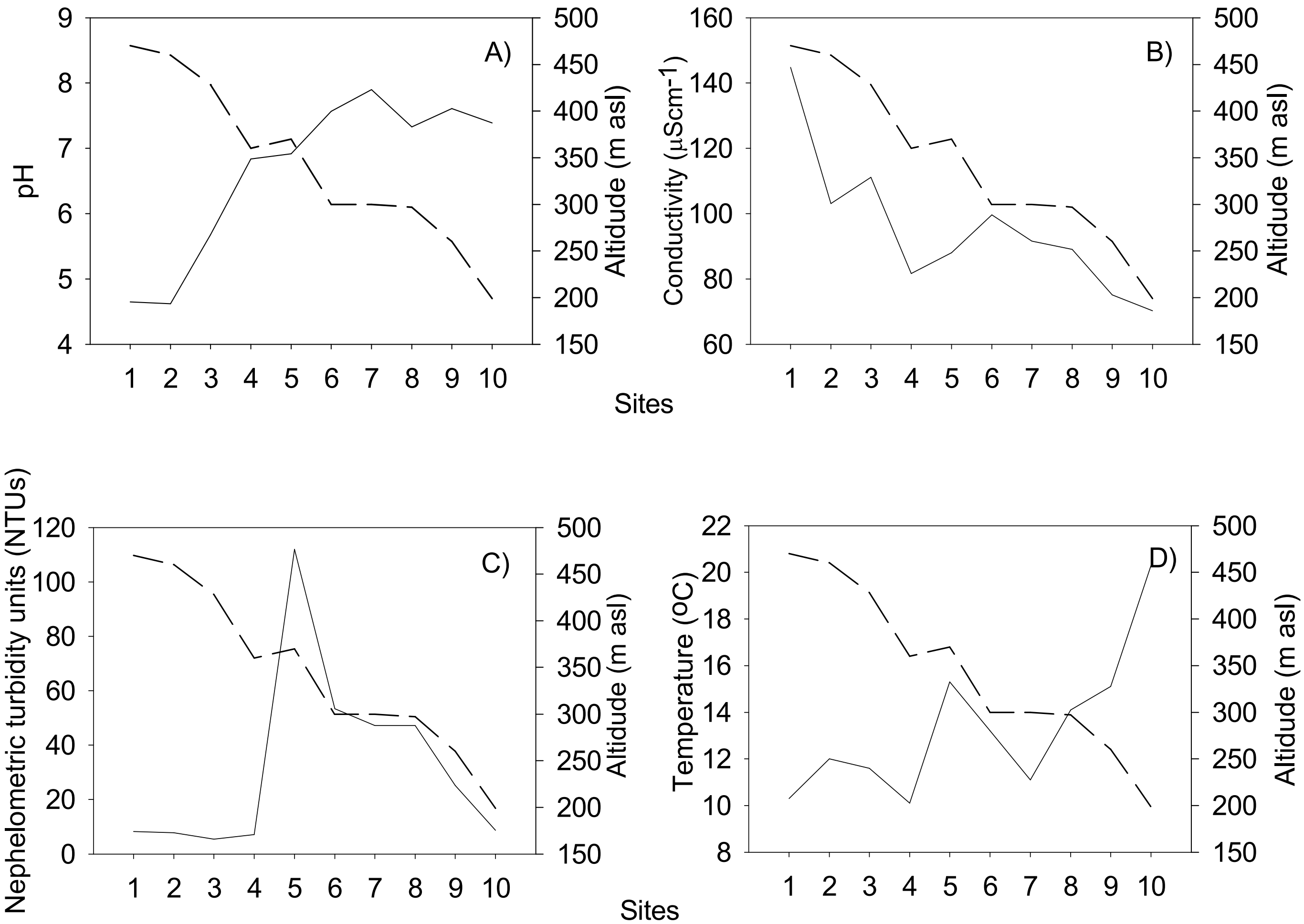

Figure 4.3. A) pH, B) conductivity, C) turbidity (NTUs) and D) temperature at each sampling station. Altitude (m a.s.l.) is shown by the dashed line relating to the right hand Y axis. The sites are: 1) Alborn car park, 2) Devils start, 3) Globe gate, 4) Devils 4, 5) Union Creek, 6) Devils (Union/Oriental), 7) Oriental Creek, 8) Progress junction, 9) Devils farm, 10) Devils highway (sampled 4 - 5 January 2007).

4.3.2. Longitudinal change in periphyton communities

Statistically none of the community metrics differed significantly with regard to either elevation or distance downstream of site 1, however some were obviously extremely variable (Fig. 4.4. B). Extensive algal cover and high biomass (the algal cover x algal depth metric) were noted both at the moderately AMD-impacted uppermost reach, and at the lowest, where the stream may be considered mildly AMD impacted and suspended solids were few. The majority of other sites had very low algal cover and biomass.

36 algal taxa were found at the 10 sites over 4 - 5 January 2007. Overall, the catchment algal flora was dominated by Chlorophyta (69% of taxa), had moderate to low diversity of Bacillariophyta (18%) and Cyanophyta (11%) and much lower diversity of Rhodophyta (1%) and Euglenophyta (<1%).

Union Creek (site 5) (112 NTUs) had low diversity and algal biomass estimates. Only a single specimen was found of each of the filamentous chlorophytes *Klebsormidium acidophilum* Novis and *Microspora quadrata* Hazen. In contrast, and despite the relatively high levels of suspended clays (47.2 NTUs), the tributary Oriental Creek (site 7) had high taxonomic richness (16 taxa) comprising 30% Cyanophyta, 13% Chlorophyta and 57% Bacillariophyta. A number of changes occurred to periphyton composition within Devils Creek. At Alborn car park (site 1), the highest altitude site, the community was dominated by Chlorophyta (99.5%) as estimated by relative abundance cell counts, while Euglenophyta and Bacillariophyta were also present. At Devils Start (site 2) the community was dominated by Chlorophyta (100%).

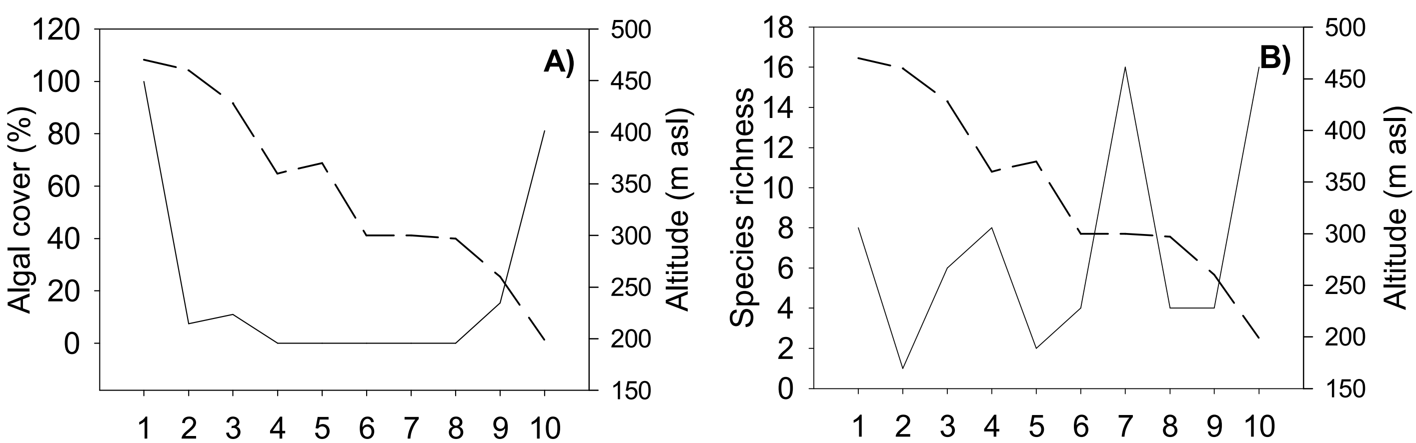

Figure 4.4. A) algal cover and B) taxonomic richness, i.e. total number of species at each of the sampling sites. Altitude (m a.s.l.) is shown by the dashed line relating to the right hand Y axis. The sites are: 1) Alborn car park, 2) Devils start, 3) Globe gate, 4) Devils 4, 5) Union Creek, 6) Devils (Union/Oriental), 7) Oriental Creek, 8) Progress junction, 9) Devils farm, 10) Devils highway (sampled 4 - 5 January 2007).

By the Globe gate (site 3) composition remained predominantly Chlorophyta (99%) with a low abundance of Bacillariophyta (1%). Some interesting changes were noted at Devils 4 (site 4) where bacillariophytes (71%) then dominated, followed by cyanophytes (23%) then chlorophytes (5%). After the confluence with Union Creek (site 5), the community composition at Devils (Union/Oriental) (site 6) was then dominated by Cyanophytes (50%) with Bacillariophytes (30%), Chlorophytes (10%) and Rhodophytes (10%) making up the remainder. Progress Junction (8) and Devils Farm (9) were both dominated by phyla Chlorophyta (99-95%), while Rhodophytes and Bacillariophytes were also present (1-5%). Devils highway (10) was composed of Chlorophytes (73%), Bacillariophytes (18%) and Cyanophytes (10%).

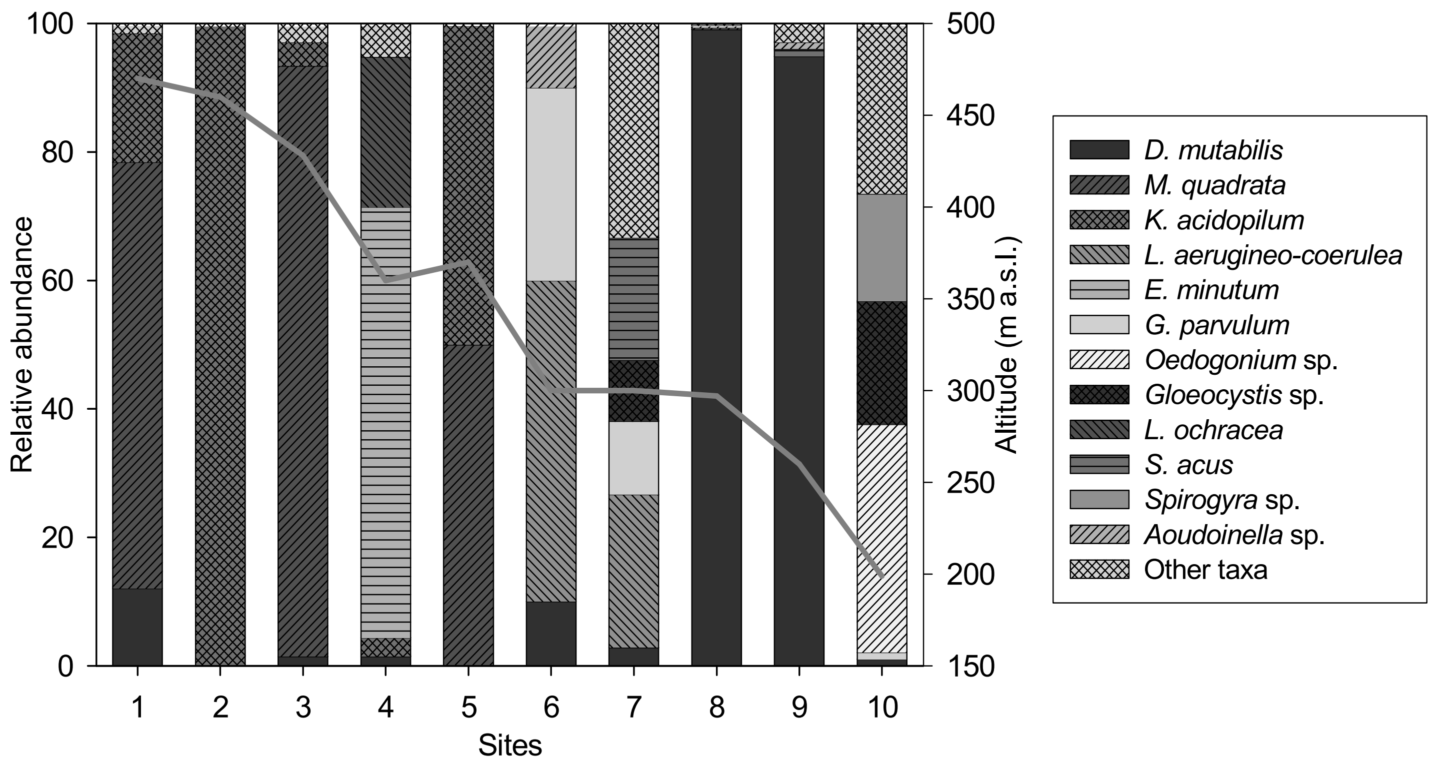

Figure 4.5. Longitudinal change in relative abundance estimated by cell counts of 12 dominant species within Devils Creek including two tributaries (Union Creek, Oriental Creek). Altitude (m a.s.l.) is shown by the dashed line relating to the right hand Y axis. The sites are: 1) Alborn car park, 2) Devils start, 3) Globe gate, 4) Devils 4, 5) Union Creek, 6) Devils (Union/Oriental), 7) Oriental Creek, 8) Progress junction, 9) Devils farm, 10) Devils highway (sampled 4 - 5 January 2007).

The dominant taxa (i.e. those that comprised more than 1% of total relative abundance) collectively comprised 92.7% of the community data set. They include: *Draparnaldia mutabilis* (Roth.) Cedergren (22.3%), *Microspora quadrata* Hazen (20.8%), *Klebsormidium acidopilum* Novis (17.7%), *Lyngbya aerugineo-coerulea* (Thuret) Gomont (7.4%), *Encyonema minutum* (Hilse ex Rabenh.) Mann (6.7%), *Gomphonema parvulum* (Kütz.) Kützing (4.3%), *Oedogonium* sp. (3.6%), *Gloeocystis* sp. (2.9%), *Lyngbya ochracea* (Thuret) Gomont (2.3%), *Synedra acus* (Kütz.) Hust. (2%), *Spirogyra* sp. (1.7%) and *Aoudoinella* sp. (1.1%).

Dominant taxa changed markedly between many sites. Upstream sites Alborn car park (1), Devils start (2) and Globegate (3) were dominated by *K. acidophilum* and *M. quadrata*. The dominant taxa within Devils 4 (4) were not present at any other site (Fig. 4.5). Union Creek (5), superficially had high abundances of *K. acidophilum* and *M. quadrata* but this is an artifact of the 'estimated relative abundance' technique, this man made stream essentially lacked periphyton. The Devils (Union/Oriental) site (6) and Oriental Creek (7) had many similar taxa such as *L. aerugineo-coerulea*, *G. parvulum* and the ubiquitous *D. mutabilis* which appeared in 80% of sites. *D. mutabilis* dominated at both Progress Junction (8) and Devils Farm (9) where it comprised 99% and 95% of composition respectively. By Devils Highway (10) the dominant organisms were again markedly different and composition was predominantly *Oedogonium* sp., *Gloeocystis* sp. and *Spirogyra* sp.

Dominant taxa and their guilds appear to be exhibiting strong altitudinal zonation, long, trailing filamentous species towards the headwaters (*K. acidophilum, M. quadrata*), changing to diatoms (*G. parvulum*, *S. acus*) and more prostrate filamentous forms (*L. ochracea*, *L. aerugineo-coerulea, Aoudoinella* sp) in middle reaches. Composition then changes giving way to species typical of stable downstream reaches (*Gloeocystis* sp., *D. mutabilis*, *Oedogonium* sp., *Spirogyra* sp.).

4.3.3. Environmental factors influencing community change

The first three of the CCA axes cumulatively explain 61.6% of the variation in the species matrix. Axis one explains the highest proportion of this variance 21.3% while axis two and three explain slightly less, 20.7% and 19.6% respectively. Axis 1 is highly correlated

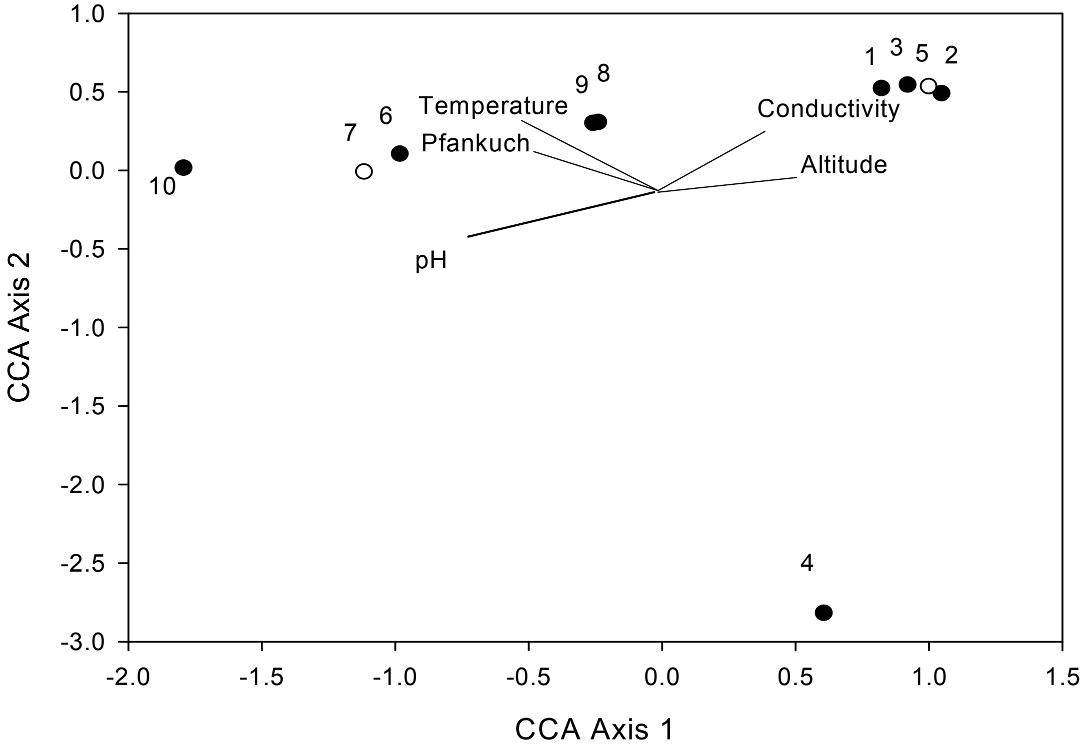

Figure 4.6. CCA biplot showing the relationship between the environmental variables and algal assemblages at each site. Sites are numbered 1-10, top to bottom, tributaries are clear points (n=10). The sites are: 1) Alborn car park, 2) Devils start, 3) Globe gate, 4) Devils 4, 5) Union Creek, 6) Devils (Union/Oriental), 7) Oriental Creek, 8) Progress junction, 9) Devils farm, 10) Devils highway (sampled 4 - 5 January 2007).

Table 4.1. CCA summary statistics and correlations with physicochemical data.

Summary statistics	Axis 1	Axis 2	Axis 3
Eigenvalue	0.95	0.92	0.88
Cumulative variance explained	21.30	42.00	61.60
Pearson correlation (Spp-Evnt)	1.00	1.00	1.00
Axis correlations (ter Braak 1986)			
pH	-0.73	-0.21	0.35
Conductivity	0.50	0.33	0.00
Temperature	-0.56	0.28	-0.37
Precipitate index	0.22	0.03	0.13
Pfankuch	-0.48	0.19	-0.33
Surface water velocity	-0.41	-0.25	-0.20
Substrate	-0.19	-0.21	0.68
NTU's	-0.01	0.25	0.24
Elevation	0.88	0.11	-0.10

with several environmental variables. Such as: elevation (0.88), pH (-0.71), temperature (-0.56), conductivity (-0.50), stream stability (Pfankuch; 0.48) and surface water velocity (-0.41; Table 4.1). Axis two is not highly correlated with any of the physicochemical variables. This indicates that variation along this axis may be due to an environmental variable not measured. Devils 4 (4) differs dramatically along this axis. None of the physicochemical measurements obtained from this site were markedly different from other sites. Several algal taxa were unique to this site and one dominated its composition, 67% of community composition was *Encyonema minutum*, 23% was *Lyngbya ochracea*, an unidentified *Gomphonema* sp. was present but in low abundance 1.5%. Substrate is also closely correlated with community variance (not graphed) and had the highest correlation with axis 3 (0.68). Sites 1 - 3 are clustered according to AMD impact, reflected in their species composition, *K. acidophilum*, *M. quadrata*, *Euglena mutabilis* Schmidtz, *Mougeotia* cf. *depressa* (Hassal) Whittrock, *Eunotia lunaris* var. *subarcuata* (Nägeli) Grunow, *Microthamnion kuetzingianum* Nägeli.

4.3. Discussion

The physicochemical variable explaining the most variation in the community data set was altitude. This was significantly correlated with a number of other variables known to influence algal assemblages, e.g. pH (Hirst et al. 2004), conductivity (Verb and Vis 2005), precipitate deposition (Niyogi et al. 1999) and clay suspensoids (Davies-Colley et al. 1992). Altitude appears to encompass the cumulative changes in response to all of: AMD, the natural longitudinal gradient and changes in suspensoids. As others have noted, where there are close correlations between physicochemical variables, elucidating the mechanism driving changes or establishing the relative roles of interacting mechanisms is difficult (Medley and Clements 1995; Verb and Vis 2005). However, given the known associations of a number of taxa (Chapter 2; 3), it appears that community composition within upper reaches of this system is primarily AMD driven.

Clay suspensoids are regarded as one of the most important of freshwater pollutants in terms of the damage they cause to aquatic ecosystems (Ryan 1991). Where they were at their most abundant, i.e. within Union Creek and directly after the confluence with Devils Creek, physical effects on the ecosystem were dramatic. In contrast to the other variables known to influence algal assemblages (natural longitudinal change, pH, conductivity), suspended clays appear to be of far lesser importance in Devils Creek for determining taxonomic composition or diversity. For example, Oriental Creek had high diversity across phyla, with a taxonomic richness of 16, but also had high suspended solid inputs (47.2 NTUs). Clay inputs did appear to suppress the estimates of algal biomass and cover, which only had high values at low turbidity. Interestingly, the large inputs of clay and surface waters entering Devils Creek at both the Union and Oriental Creek tributaries appears to have counteracted the effects of AMD over a short distance. Where clay inputs first enter Devils Creek, pH becomes basic increasing above pH 7, presumably due to base anions neutralizing acidity. Conductivity also decreases and may be explained through inorganic adsorption by suspended and settled clays (Gier and Johns 2000; Younger et al. 2002)

Medley and Clements (1998) studied longitudinal changes in periphyton assemblages within streams impacted by heavy metals. They found that assemblages in metal polluted streams were dominated by *Achnanthes minutissima* and *Fragilaria vaucheriae*, noted as early successional species. The relative abundance of these organisms decreased as metal pollution decreased. In contrast *Diatoma vulgare* and *Melosira varians* were dominant in low elevation sites and were quickly eliminated (after 24 h) when exposed Cd, Cu and Zn. They suggest that morphological and life-history characteristics of these diatoms influence their tolerance to metals, i.e. characteristics that allow a species to dominate early successional stages also allow tolerance to metals. No explanation was given regarding the mechanism of this pattern (Medley and Clements 1998) and this pattern was not apparent in the present study. The adnate *Achnanthes* spp. were more common downstream. Other diatoms that may fill a similar niche were present in mid-reaches, e.g. *E. minutum*, *G. parvulum*. In contrast, and similar to the findings of this study, Hill et al. (2000a) found diversity did not significantly increase with distance downstream, as mining impacts lessened. Hill et al. (2000a) found changes in taxonomic composition of periphyton assemblages, related to the degree of metal contamination. *Fragilaria* sp. dominated less impacted, while *Achnanthes* sp. dominated more impacted sites.

Despite the fact that AMD and clay inputs are subsumed within altitude it appears that both have an interacting effect on the community. Physicochemical stressors of upstream mining activities rapidly decreased, which was reflected in the algal community, where taxa indicative of AMD pollution including the abundant acidophile *K. acidophilum* and the more broadly tolerant *M. quadrata* disappear within 2.2 km of AMD inputs. Periphyton recovery appeared to be occurring within 5km of AMD and clay inputs. Devils Highway did not have suppressed estimates of algal cover or biomass due to clay inputs, had high taxonomic richness and had taxa identified earlier (e.g. Table 2.4) to be intolerant to AMD stress. These included: *Heteroleibleinia purpurascens* (Hansgirg ex Hansgirg) Anagnostidis & Komárek, *Achnanthes oblongella* Østrup, *Oedogonium* sp. and *Spirogyra* sp. the latter a typical genera of many larger NZ waterways (Biggs 1990).

Strong altitudinal zonation reflected in algal guilds also appeared to be occurring, similar to the findings of other authors (Ward 1986; Molloy 1992; Passy 2007). The importance of naturally varying factors on taxonomic composition and guild structure becomes apparent in middle reaches. Naturally varying factors that may be of particular importance within Devils Creek include, canopy cover, substrate type, grazing pressure and the varying effects of flow and disturbance regime in relation to how constrained a particular reach is. As stated earlier a biological guild may be defined as a group of species that exploit the same class of environmental resources in a similar way (Root 1967). However, in this instance and again similar to the findings of Molloy (1992), the change of guilds appears to be defined by the influence of flow and disturbance. In contrast to Molloy (1992), in upper reaches trailing filamentous forms were noted, although this may be explained by stable AMD conditions (Chapter 1). Sites 4, 6 and 7 were the middle reaches, and were the steepest, most constrained and therefore probably the most disturbed. These factors may explain the strong divergence of site 4 along axis 2 of the CCA, and the failure of the analysis to account for this divergence where channel constraint, stream gradient and disturbance were not measured. Inherent community patchiness may also contribute to this result however (Pringle et al. 1988; Townsend 1989). Taxa and guilds within these reaches sites 4, 6, 7 appeared to be influenced primarily by disturbance, channel constraint, gradient and flow. Algal guilds present were indicative of these conditions, and dominant organisms included stalked (*G. parvulum*) and adnate diatoms (*E. minutum*), and prostrate filamentous algal forms (*L. ochracea*, *L. aerugineo-coerulea, Aoudoinella* sp.). In later reaches that were less steep and constrained, dominant algal guilds reflected this, where large trailing growths of *D. mutabilis*, *Oedogonium* sp. and *Spirogyra* sp. were present.

Chapter 5: Taxonomy of dominant algae in heavily impacted AMD

Numerous studies have found that chlorophytes usually dominate AMD. Species of *Klebsormidium*, *Microspora*, *Mougeotia*, *Ulothrix, Zygnema, Stigeoclonium* and *Microthamnion* among others have all been noted previously (e.g. Bennet 1969; Graham 1996, Niyogi 1999; Niyogi et al. 2002; Sabater 2003) in very low pH AMD streams (Chapter 1, 2). Other taxa recorded include several species of diatoms and, commonly, *Euglena mutabilis* (Chapter 1, 2, 3 and 4).

In this study, several algae were common inhabitants of severely and moderately impacted streams and may be useful 'tolerance' indicators (*sensu* Fore and Grafe 2002). It is these taxa which are described below. They are: the naviculoid diatom *Navicula cincta* (Ehrenberg) Ralfs, the eugleophyte *Euglena mutabilis* Schmidtz, and the chlorophytes *Klebsormidium acidophilum* Novis, *Microspora* cf. *quadrata* Hazen and *Microthamnion kuetzingianum* Nägeli.

The following provides a description of specimens observed during this study and notes on their ecology in AMD waters. Classifications are based on Krammer Lange-Bertalot 1991a; 1991b and John et al. 2002.

Phylum: BACILLARIOPHYTA

Order: Naviculales

***Navicula cincta* (Ehrenberg) Ralfs**

Description: Valves variable, oblong elliptic to slightly lanceolate, ends bluntly rounded; 5-7 µm wide typically 5 µm; 15-40 µm long typically 20 µm. Numerous striae that radiate strongly in the central area, becoming more closely parallel. Absent from a central circular area in the centre of the valve. Large oil vacuoles present in healthy specimens. Paired, parietal golden-brown chloroplasts opposite when engorged occupying 95%, usually offset and opposite occupying >90% of cell length. Motile although movement very gradual, positively phototactic (Fig. 5.1. E,F).

Remarks: According to DeNicola (2000) *N. cincta* has not been described from AMD habitats before. In New Zealand, it has been noted by Biggs and Kilroy (2000) to be common in high conductivity streams. It was present at pH 2.9 - 4.4 (mean 4.3) and at conductivities of 428 - 1220 μ S cm^{-1} (mean 876 μ S cm^{-1}) in sites with moderate iron hydroxide deposition. It formed epipelic (precipitate dominated substrate), episammic and epilithic dark brown mats.

Phylum: EUGLENOPHYTA

Order: Euglenales

***Euglena mutabilis* Schmidtz**

Specimen features: Cells 4-20 µm wide, 30-70 µm long, length tapering dependant upon movement. Cylindrical, elongate spindle shaped at full extension. Anterior end narrowing to a tip, posterior end blunter. Stigma orange-red central to anterior. Numerous chloroplasts ~4, parietal, bright green/yellow green typically occupying 75-80% of the cell lumen. Pyrenoid difficult to resolve with light microscopy. Paramylon often densely packed, short rectangular bodies. Flagellum not visible. Motilty moderate. Red-orange granules present within some specimens (Fig. 5.1. A,B).

Remarks: Possibly the most commonly described AMD alga (Chapter 1, 2). It was present at pH's ranging from 2.7-6, with a mean value of 3.8. It was found at conductivities between 39 -1220 μ S cm^{-1}, with a mean value of 477 μ S cm^{-1}. It was found at sites that had moderate to high levels of precipitate deposition, and was noted at both stable and hydraulically disturbed sites and was noted as forming epipelic (precipitate dominated substrate), episammic and epilithic (characteristic of disturbed sites) light to dark green mats.

Phylum: CHLOROPHYTA

Order: Klebsormidiales

***Klebsormidium acidophilum* Novis**

Specimen features: Filaments uniseriate, unbranched. Mucilage pads common. Cells size variable dependant on the environmental conditions where the sample was collected from. Cells cylindrical although may appear barrel-shaped with constrictions at the cross wall in concentrated AMD. Filaments are 5-8 µm wide, typically 6 µm and 5-18 µm long, typically 8. Cell walls thin. Chloroplast, parietal, single, ellipsoidal girdle shaped and with a smooth margin, encircling 50% of cell lumen with a single distinct pyrenoid (Fig 5.1. C)

Remarks: A recently described alga that commonly forms extensive proliferations in AMD habitats (Novis 2006; Chapter 2), and has probably been identified as *Klebsormidium* sp., *K. rivulare or Ulothrix* sp. in numerous previous studies (Chapter 1). It was present at pH's ranging from 2.7-6, with a mean value of 3.8. It was found at conductivities between 70 – 1220 μ S cm^{-1}, with a mean value of 445 μ S cm^{-1}. It was found at sites that had moderate to extremely high levels of precipitate deposition, and was present at both stable and hydraulically disturbed sites. Bathhouse stream (pH, 2.9; conductivity 1200 μ S cm^{-1}) on the Stockton Plateau appeared to be a very stable, constrained AMD stream that had consistently high water velocities (0.34 m/s recorded for the survey and noted during preliminary sampling). Within reaches of this stream that did not have heavy canopy cover, prolific green algal growths were noted on several occasions, which were dominated by *K. acidophilum*.

Order: Microsporales

***Microspora quadrata* Hazen**

Specimen features: Filaments uniseriate, unbranched, without basal or apical differentiation. Cells cylindrical to slightly barrel shaped with slight cross wall constrictions, 6 - 9 µm wide, (2-) 4 – 9 µm. Chloroplast, parietal, reticulate, completely surrounding cell lumen, but with numerous lobes and perforations. Cell walls ≤ 1 µm thick, often with visible extra H shaped thickenings, occasionally forming extensive yellow-brown lengths of rough thickening. Between cell sections thin, not thickened (Fig. 5.1. D).

Remarks: A recently taxonomic survey of New Zealand *Microspora* allows species identification from vegetative structures. At least one other study has reported *Microspora* from AMD, but identified the species as *M. tumidula,* morphologically a very similar species.

Although identification notes on *M. quadrata* (Novis 2004) state this species often has an extra discontinuous thickening, that was present on specimens here and was a feature not present in the notes of *M. tumidula* (John et al. 2002). It was present at pH's ranging from 2.9 - 7, with a mean value of 4.5. It was found at conductivities between 24 – 1200 μ S cm^{-1}, with a mean value of 310 μ S cm^{-1}. It was found at sites with no precipitates and at sites that had very high levels of precipitate deposition. It was most common at stable moderately impacted AMD sites and was noted to form dark green epilithic filamentous growths.

Order: Microthamniales

***Microthamnion kuetzingianum* Nägeli**

Specimen features: Filaments densely and irregularly divided in a very characteristic manner, spreading. Cells cylindrical, curved or straight, 3-4 µm, typically ~3 µm wide and 6 - 65 µm long, typically ~40. Apical cells bluntly rounded, basal cell a differentiated semicircular attachment cell. Chloroplast is light green, single, thin, with a smooth margin and is parietal, occupying ~25% of the internal cell circumference, running down 25% to 95% of the cell length (Fig. 5.1. G,H).

Remarks: This is a species previously recognized from AMD and is cited as frequently occurring on iron and manganese hydroxide deposits (John et al. 2002) and was positively associated with iron hydroxide deposition noted in this study (table 2.4). It and was present at pH's ranging from 2.9 - 7.1, with a mean value of 4.3 and was found at conductivities between 39 – 1220 μ S cm^{-1}, with a mean value of 373 μ S cm^{-1}. It was a species that rarely appeared to have macroscopically visible growths.

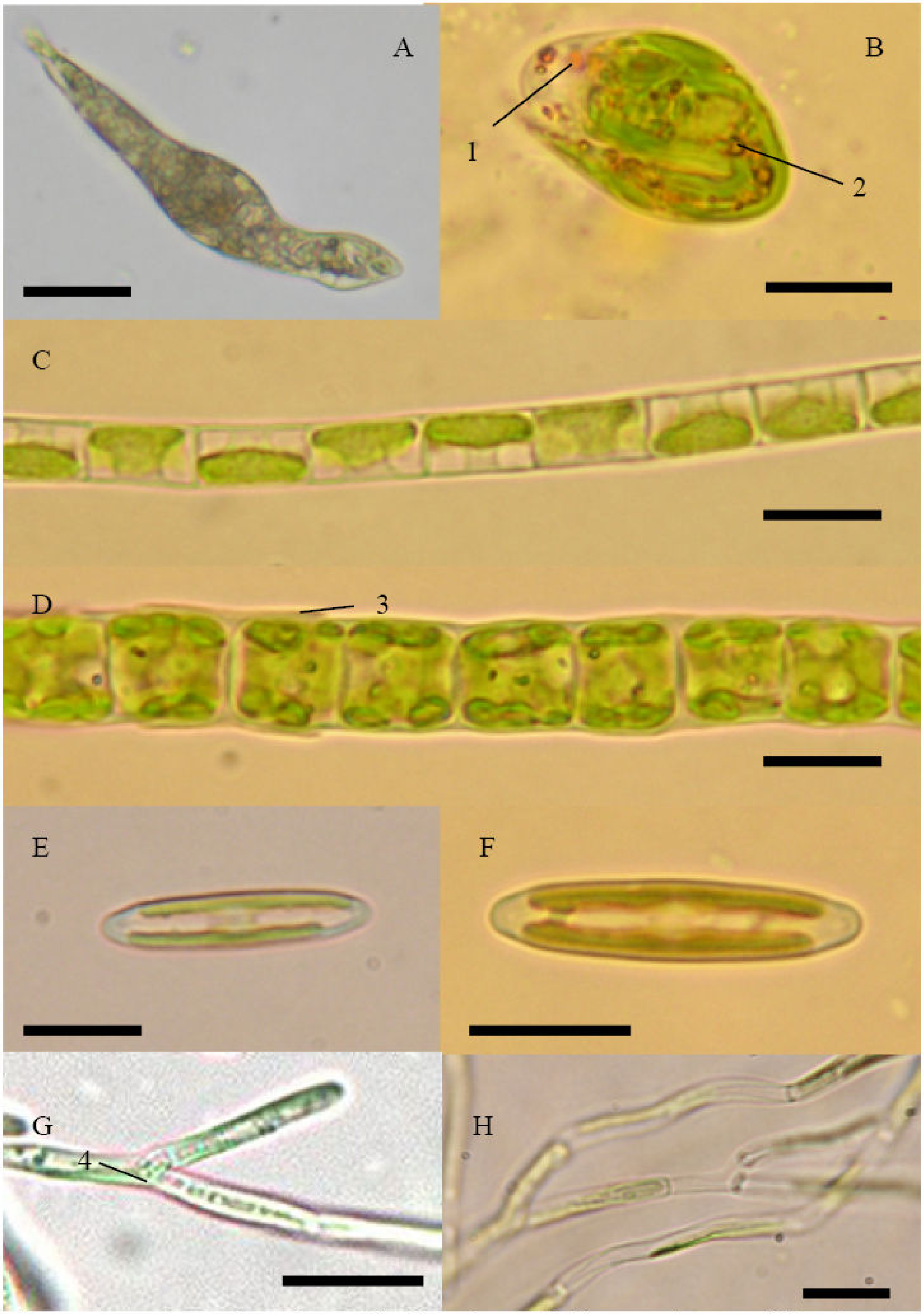

Figure 5.1. Dominant algae of severely affected AMD habitats. A,B) *E. mutabilis,* C) *K. acidophilum,* D) *M. quadrata,* E,F) *N. cincta,* G, H) *M. kuetzingianum.* Characteristics, 1) stigma, 2) iron deposits, 3) H shaped cell wall thickening, 4) branching patten. Scale bars all 10 um.

Chapter 6: General Discussion

6.1. Periphyton assemblages across spatio-temporal gradients

Periphyton is influenced by a wide range of biotic and abiotic factors (Chapters 2, 3 and 4). Spatially these factors act on both macro- and micro-habitat scales, influencing both benthic algal structure and function (Biggs 1990; Biggs and Gerbeaux 1993; Biggs et al. 1998). However, AMD and its associated stressors may be considered a 'sledgehammer blow' (sensu Schindler 1987) and of overriding importance to the ecosystem in question (Harding and Boothroyd 2004).

This study shows how changes in physicochemical variables associated with AMD influence benthic algal taxonomic composition and has marked negative impacts on diversity over broad scales. This finding is in accordance with some studies (Verb and Vis 2005) and in contrast to the findings of others (Deniseger et al. 1986; Hill et al. 2000a), where other measures of diversity have been used, or where fewer streams have been compared. Low diversity, as indicated by indices, may be accounted for by two mechanisms in stressed systems, a low number of taxa or very high dominance by one or a few taxa (Niyogi et al. 2002) and often fail as useful indicators of stress (see section 2.4.1).

AMD generally increases taxonomic dominance and causes increases in algal cover and biomass. Algal cover and biomass were found to be significantly higher in the most severely affected streams, in accordance with some authors but not others (see Chapter 1; 2). Thus algal biomass in West Coast AMD streams may be influenced by: the severity of AMD; the rate of iron oxide deposition (rather than aluminium oxide; Niyogi et al. 1999); hydraulic disturbance (Biggs 1996; Biggs and Smith 2002) and the extent of grazing release (Rosemond et al. 1993; Winterbourn 1998; Anthony 1999; Nyström et al. 2003). Each of these factors needs to be considered before conclusions are drawn about the affects of increasing acidity on algal biomass within streams.

Community change across an AMD gradient has been shown to be predictable (Verb and Vis 2000; 2005). On the West Coast it appears severely AMD impacted communities and moderately impacted communities are relatively predictable, where a limited number of taxa may be present and losses of these taxa are observed with decreasing stress. Predicting an end state or detecting periphyton assemblage responses, appears to become more difficult the lower the level of stress a community is subject to. This is because communities become increasingly more diverse and variable (Chapter 2) and impacts are far more subtle. Detecting community responses where stresses are minimal has long been recognized as a difficulty facing biomonitoring (Schindler 1987). Only one other algal survey, conducted by Verb and Vis (2005), surveys across such a wide spatial scale, incorporating multiple catchments and includes a wide gradient of mine-stressed streams. The species-based models developed by Verb and Vis (2005) were highly predictable for pH, indicating they may be useful for evaluation of coal mine remediation.

The time of year was identified during the broad scale survey as a major factor influencing periphyton assemblages (Chapter 2). Seasonal changes are accepted to occur within algal communities of AMD streams (Deniseger et al. 1986; Verb and Vis 2001; 2005) and streams unaffected by stressors (Sheath and Burkholder 1985; Oemke and Burton 1986) and both were observed in this study. It appears that within AMD streams these changes were less pronounced because AMD stressors initially constrained the number of species capable of growth and therefore change. Temporal change occurring within reference and mildly affected communities was more marked. Numerous taxa, including many that were of low abundance, changed according to the month of sampling.

In this study severely affected AMD habitats (and many moderately affected) tended to be smaller in size and their main (or only) source was often a mine adit. These adits appeared to have very stable flow (therefore disturbance) regimes. I would hypothesize, where little ground water dilution can occur, they may also have stable water chemistry conditions. Other authors have also noted that these systems, due to their infrequency, size, placement and harsh physicochemical conditions, may have few new sources of colonists (Gross 2000). These factors in addition to those discussed above may promote high biomass and consistent community composition.

Longitudinal changes in algal assemblages in Devils Creek appear to be predictable where the degree of AMD stress was high within upper reaches. AMD stressors collectively weakened with distance downstream or altitude as may be expected where dilution, sorption and precipitation improve water quality (Younger et al. 2002). In Devils Creek, it further appeared that the suspended sediment inputs may counteract the effects of AMD. This may be because base anions are present in the opencast effluent neutralizing acidity and creating basic conditions. High concentrations of inorganic matter can also adsorb metal ions, shown in other studies (Farrah and Pickering 1977). Once this occurs, it appears AMD is a lesser influence on algal communities and zonation due to natural factors appears to be significant within Devils Creek. Periphyton composition, physical structure and associated guild changes were noted and appeared to be in direct response to changes in stream stability, surface water velocity, stream gradient and how constrained the channel was. Periphyton guild and structure changes are recognized to occur down stream lengths (Ward 1986; Molloy 1992; Passy 2007).

6.2. Surveying AMD impacts on benthic algae

This study surveyed benthic algal communities primarily across a broad AMD gradient, while also attempting to account for other broad scale controlling factors such as the effect of temporal and longitudinal change. Experimental manipulations such as bioassays (von Dach 1943), mesocosm (Perrin et al. 1992; Bortnikova et al. 2001) and microcosm (Anthony 1999) experiments, or field manipulations (Niyogi et al. *In press*) are powerful tools for investigating organism and community responses to stressors. However, few ecological certainties may be made unless biomonitoring surveys are conducted to supplement this data (Griffith et al. 2002). Biomonitoring surveys collect complex data, but with the use of multivariate statistical analyses, the primary stressors influencing community responses may be elucidated at the broadest scale which is the most relevant for environmental management (Omernik 1995; Griffith et al. 2002).

6.3. Periphyton as a tool for monitoring AMD impacts

Periphyton is ubiquitous, abundant, diverse and an important ecological component of lotic environments (McCormick and Stevenson 1998). It is an important base of food webs in many New Zealand waterways, where it is used as a food source by many invertebrates (Biggs et al. 1998; Winterbourn 2004). Periphyton is also an important structural component of lotic ecosystems, stabilizing the substrate while providing refugia for fish and invertebrates (Bott 1996). Benthic algae are also sensitive to changes in water quality and can respond rapidly and predictably to a range of environmental conditions associated with AMD, such as pH and dissolved metal concentrations (Hirst et al. 2004). Benthic algae have short generation times allowing them to respond rapidly to changes in water quality (McCormick and Stevenson 1998). They are also sedentary, therefore directly indicative of the physicochemical conditions of their immediate surroundings and of the catchment. In contrast, invertebrate drift or fish movement may be more likely to confound biomonitoring results where these organisms are used. Where stresses are severe fauna may also be generally less tolerant e.g. AMD (Harding and Boothroyd 2004). As has been shown here, AMD stressors are a major factor driving algal community composition and productivity. A wide variety of algae tolerate the often severe conditions of AMD and this may give a higher degree of resolution in severely affected streams than may be obtained from other indices, where for example macroinvertebrates may be completely excluded. Diatoms may also be used in certain circumstances to provide benchmarks of historical water quality conditions (Stoermer and Smol 1998). Because of the diversity of benthic algae and the wide ranging sensitivities of different taxa, algae may also be ideally suited for characterizing the minimally impacted biological condition of marginally disturbed ecosystems (McCormick and Cairns 1994). Quantifying contaminants by chemical analyses, e.g. dissolved metals, is expensive and may not be suitable for regular monitoring, and may not be accurate where water chemistry fluctuations are known or suspected (Chapter 3; Verb and Vis 2000; 2005).

These reasons may make a periphyton index of biotic integrity (PIBI) more suited to biomonitoring AMD in freshwaters than fish or invertebrates and may be a relatively rapid and cheap alternative or supplementary method (McCormick and Cairns 1994; Fore and Grafe 2002; Passy et al. 2004). Several algal tolerance indices relating to specific stressors have been generated for European streams (Prygiel and Coste 1993; Pyrgiel et al 1997; Kelly and Whitton 1998; Stevenson and Pan 1999) and it has been recognized previously that algae may be used as indicators of heavy metal and AMD stress (Hill et al. 2000a; 2000b; Verb and Vis 2000; 2005). A PIBI for use in New Zealand streams, as has been done else where, would however also need to measure the impacts of other stressors such as: agriculture; opencast and alluvial mining; heavy metals; sewage and deforestation (Pyrgiel et al. 1997; Fore and Grafe 2002).

6.4. A hypothesis regarding the role of acidophilic algae in creating and sustaining AMD

It may be possible that algae play an indirect role in maintaining the low pH, high dissolved metal conditions of AMD. Acidophilic algae have been noted to contribute to over-saturation of oxygen by up to 200% (Brake et al. 2001). The primary path of oxidation of Fe^{2+} to Fe^{3+} is bacterial oxidation, which is influenced by oxygen concentrations. Espańa et al. (*in press*) found that initial dissolved oxygen concentrations within effluents emerging from mine adits in Odiel Basin, Huelva, Spain were usually very low (< 1 mg L^{-1} DO), but markedly increased in downstream reaches (4-7 mg L^{-1} DO) due to high rates of photosynthesis in algal biofilms. They state that these high levels of dissolved oxygen among

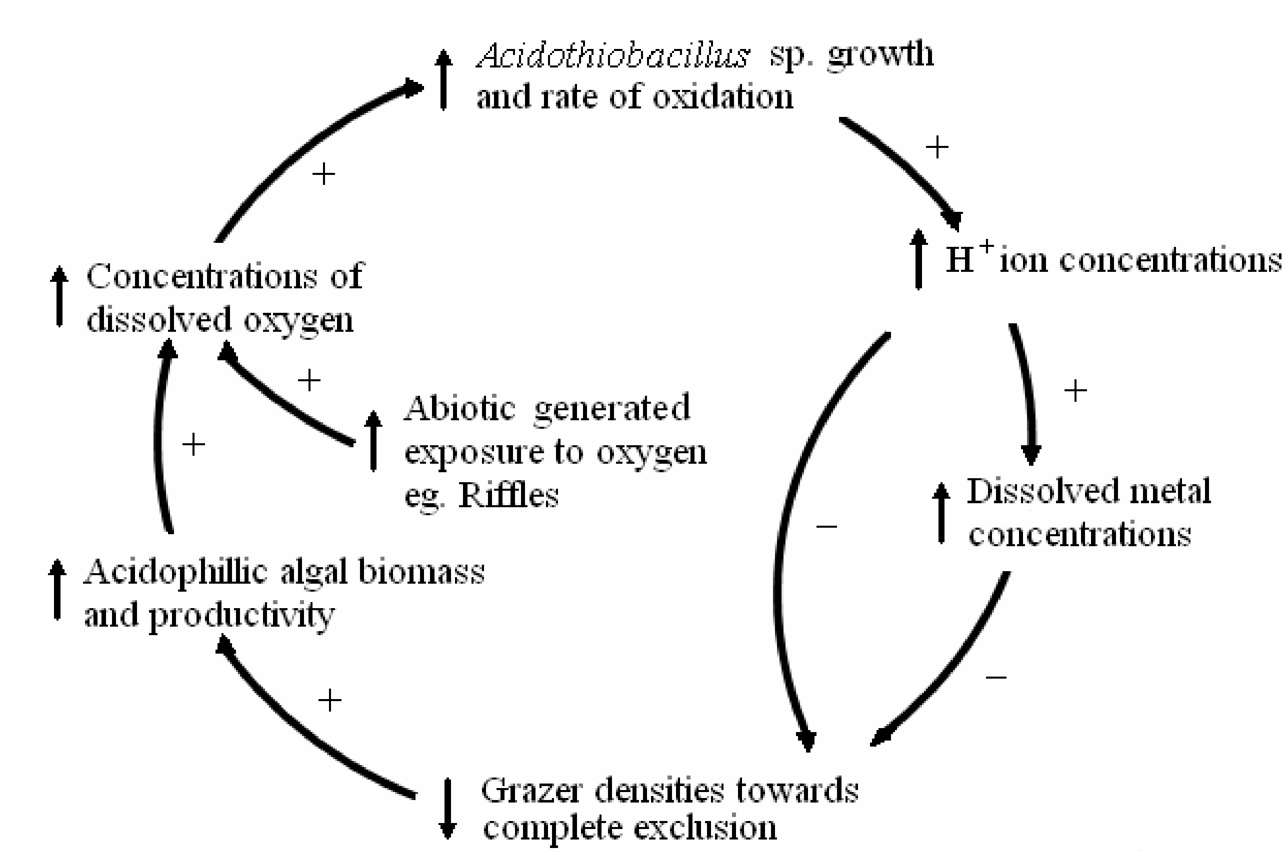

Figure 5.1. Hypothesised cycle outlining the potential role acidophilic algae play in creating and maintaining AMD.

other factors (bacterial concentrations, bacterial growth rate, temperature, pH), are the major variables affecting oxidation rate. Importantly it is this oxidation of Fe^{2+} that causes such dramatic reductions in pH (Younger et al. 2002). Where pH is reduced, it has the added effect of dissolving and maintaining metal ions in solution (Younger et al. 2002).

Dependant upon the metal, its species and the hydrogen ion concentrations the resultant effluent can have drastic effects on invertebrate grazers. Often grazers are excluded or are reduced in density to the point they are functionally absent (Winterbourn and McDiffet 1996; Winterbourn et al. 2000; Harbrow 2001; Harding 2005). This has led numerous authors to hypothesise that release from top down control may be a dominant reason for algal proliferations in low pH habitats (Graham et al. 1996; Niyogi et al. 1999; 2002; Bradley 2003). Where other factors do not negatively influence algal biomass and productivity (e.g. disturbance), the hypothesised cycle may perpetuate.

I believe this cycle may play a significant role in maintaining acidic conditions and hastening breakdown of pyrite, thereby maintaining algal communities in a down stream manner of sunlit, lotic AMD. It may also be as, or more important to the maintenance of AMD in lakes. Less mixing may create oxygen deficiencies in which bacterial oxidation and thus acid production is rate-limited by the photosynthetic activity of primary producers. This hypothesis needs thorough testing before conclusions are drawn.

6.5. AMD tolerant guilds

The mechanisms by which algae may tolerate AMD are many (section 1.4.4). In this study, observations both in the field and microscopically of AMD taxa agree with some of the findings reviewed by Gross (2000).

Of the filamentous species, *M.* cf. *quadrata* often creates thick cell coverings, which may help to maintain necessary internal cellular ion concentrations. From field observations *K. acidophilum* can occur in areas of high precipitate deposition, however within these sites it appears to grow most prolifically in sunlit, shallow, fast-flowing habitats. These situations are presumably where atmospheric CO_2 is not limiting due to mixing conditions, and where high water velocities reduce settlement of precipitates (Younger et al. 2002) which could smother growths. That occurrence of proliferations appeared to be restricted to well sunlit areas, which may seem intuitively obvious where light controls primary production, however photoreduction of iron oxides may be equally or more important where deposition rates are high (Tate et al. 1995).

E. mutabilis is moderately motile (John et al. 2002), and *N. cincta* also exhibited very slow movement, a characteristic of this genus (Fore and Grafe 2002). Motility may characterise a guild of algae which are directly associated with sediments in AMD. These unicellular, motile species live on the upper surface and within the top few millimeters of sediments. Motility would allow them to maintain their place in these superficial layers even where deposition rates are high. Motility may also allow these algae to best utilize resource heterogeneities, such as changing light or nutrient patches (Gross 2000).

6.6. Further work

Where the general survey has suggested that pH may be a factor of overriding importance, its effects need to be tested and confirmed in manipulative experiments. The pH and metal tolerances of a number of algae through species autecology work involving where possible *in situ* and *in vitro* manipulations would benefit, especially if steps towards an algal index were made for monitoring AMD impacts.

Reasons for temporal change in algal assemblages and abundance are poorly understood in New Zealand streams, although much work has been done on disturbance due to spates which are likely the primary driver. AMD impacts on taxonomic composition, particularly on certain taxa, may be especially important for selection of indicator species for use within an index. For example, where certain species have been identified as particularly sensitive or tolerant, it may be necessary to know how sensitive these species are to factors relating to temporal change, such as temperature and hydraulic disturbance.

Further work towards establishing community longitudinal gradients in New Zealand streams, would further benefit towards creation of a PIBI. Ideally a study into the longitudinal recovery of periphyton down the length stream, may involve an AMD stream with gradually recovering water chemistry and would compare a reference stream of similar geographic, geological and hydraulic characteristics. Furthermore, both streams should be completely unaffected by other anthropogenic stressors. This component was confounded by clay inputs (realized after the study was begun), and knowledge on the effects of clays on periphyton would benefit, where they are poorly known in New Zealand and internationally.

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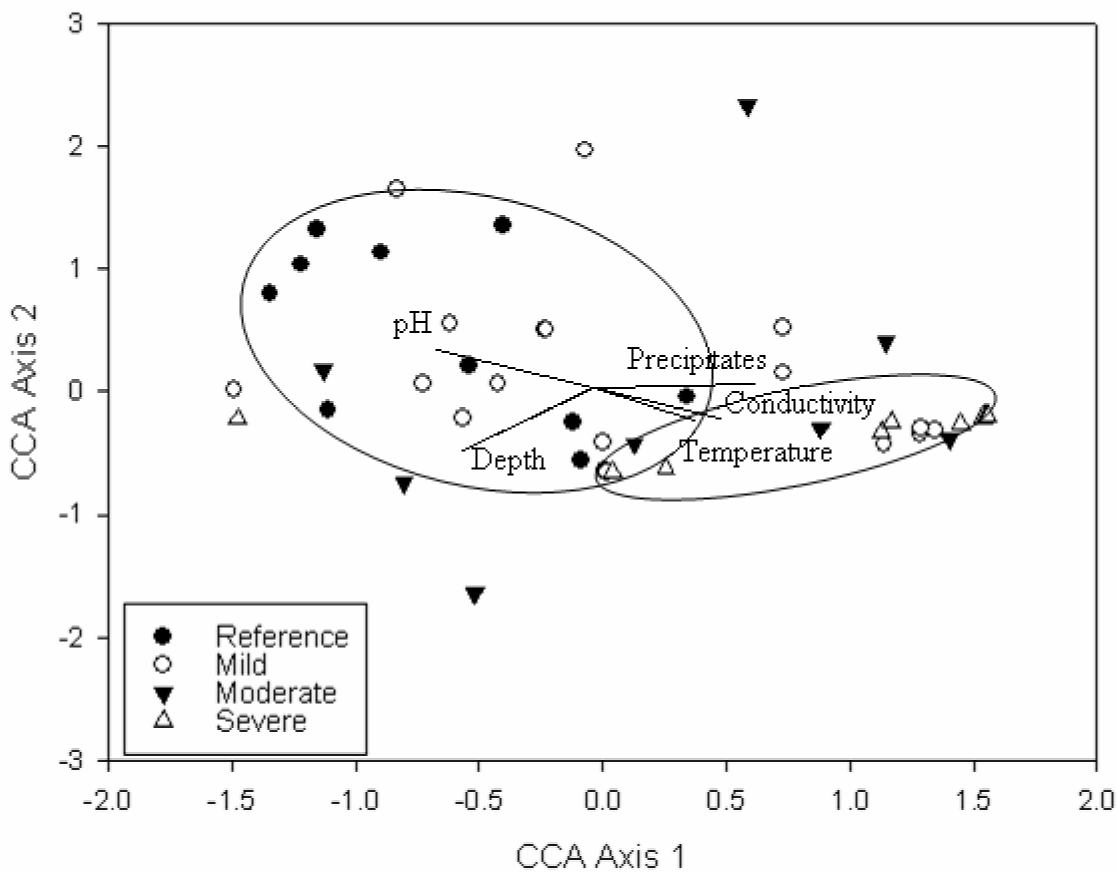
Appendices

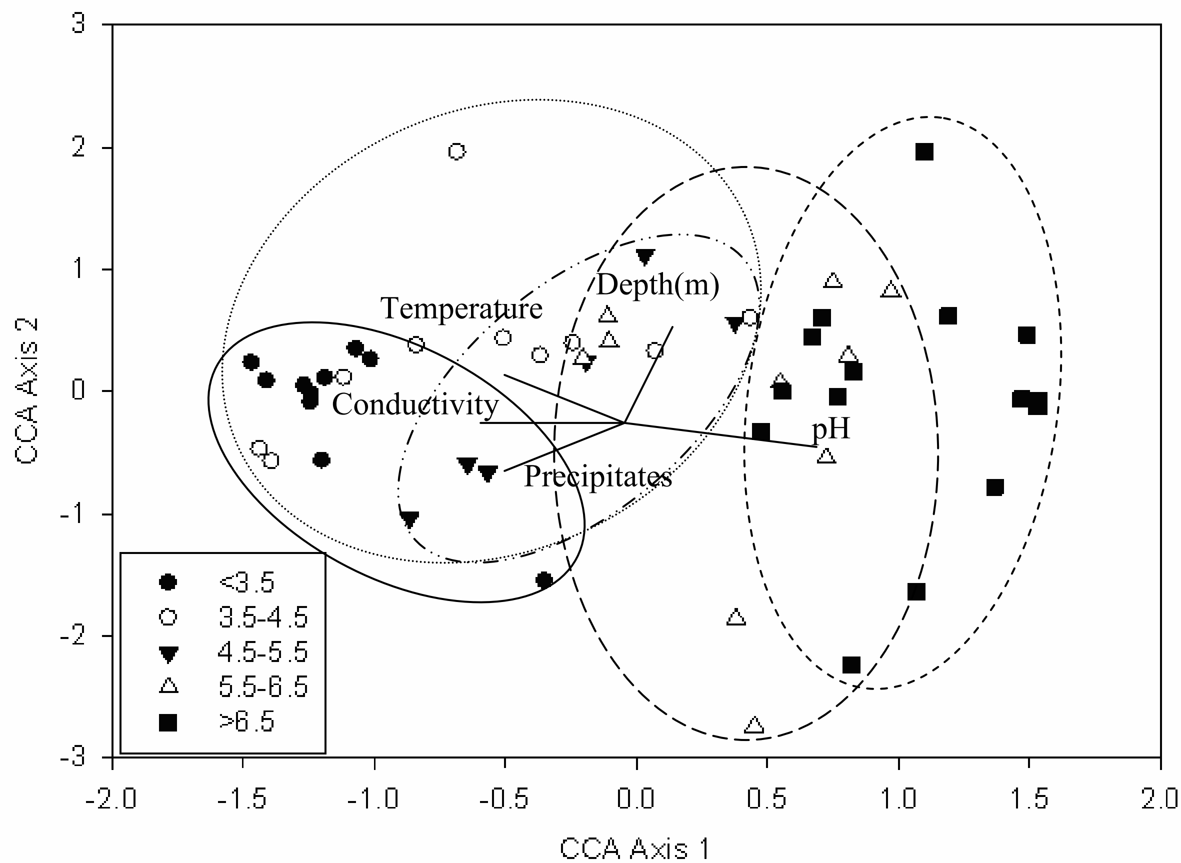
Appendix 1. Sites surveyed across a gradient of AMD stress on a single occasion between April 2006 and January 2007 (n=52). Impact categories were decided by a cluster analysis of water chemistry.

Site name	Geographic location	pH	Conductivity (μScm^{-1})	Precipitate index	Impact category	E	N
Denniston Reference	Westport	4.2	18	1	Reference	2409250	5940250
Coalbrookedale Upper	Westport	4.4	40	2	Reference	2410195	5937566
Carton Creek	Reefton	5.5	31	1	Reference	2414861	5894797
Hutt Stream	Westport	5.7	26	1	Reference	2412215	5938750
Slab Hutt Creek	Reefton	6.4	24	1	Reference	2410137	5893826
Inanguahua Trib. 1	Reefton	6.4	28	1	Reference	2421686	5891581
Carldiers Creek	Reefton	6.4	40	1	Reference	2415186	5896636
Sapling Stream	Reefton	6.7	35	1	Reference	2419020	5896660
Madman	Reefton	6.9	38	3	Reference	2418260	5900950
Coal Street Creek	Reefton	6.9	38	1	Reference	2416296	5898923
Scotchman Creek	Reefton	7.3	42	1	Reference	2420820	5893683
Locked Gate Creek	Westport	7.5	43	1	Reference	2411655	5938415
Ropers Hotel	Westport	7.6	31	1	Reference	2410971	5938324
Coalbrookedale 2	Westport	3.0	107	4	Mild	2410186	5937588
Lake Mine Seepage 1	Westport	3.1	70	2	Mild	2409190	5936990
Lake Mine Seepage 2	Westport	3.1	70	2	Mild	2409190	5936990
Mine Creek 2	Westport	3.8	127	3	Mild	2417034	5952202
Burnetts Face Stream	Westport	3.9	62	2	Mild	2410980	5938400
Roa Road Creek	Blackball	4.0	127	2	Mild	2378899	5870512
Cementown Bridge	Reefton	4.3	61	1	Mild	2419855	5896460
Gannons Road Creek	Reefton	4.5	80	2	Mild	2419895	5902490
Brunner Creek	Greymouth	4.8	93	1	Mild	2372264	5862534
Burkes Creek	Reefton	6.1	64	5	Mild	2417570	5899351
Globe/Progress Junction2	Reefton	6.3	62	1	Mild	2416715	5893827
Pyramid Creek	Reefton	6.7	85	5	Mild	2418670	5901060
Lankeys creek	Reefton	6.8	97	1	Mild	2419402	5895285
Stoney Batter Creek	Reefton	6.9	62	3	Mild	2418060	5900870
Rapahoe Stream	Greymouth	6.9	110	1	Mild	2366479	5866987
Brunner Reference	Greymouth	7.0	59	1	Mild	2371772	5861664
Ten Mile Second Trib	Greymouth	7.1	111	1	Mild	2368957	5872750
Stoney Reference Trib.	Reefton	7.1	73	1	Mild	2419745	5895135
Ten Mile Creek	Greymouth	7.3	85	1	Mild	2367958	5872687
Burkes Reference	Reefton	7.5	56	1	Mild	2417815	5899400
Coalbrookedale 1	Westport	3.5	250	4	Moderate	2410194	5937568
Wellman	Reefton	4.6	298	5	Moderate	2423011	5893613
Alborn Carpark Creek	Reefton	5.0	184	5	Moderate	2417382	5891158
Ford Creek	Blackball	5.1	155	2	Moderate	2378805	5870094
Globe closed gate creek	Reefton	5.8	149	1	Moderate	2417461	5891728
Alborn Wetland Creek	Reefton	6.0	241	2	Moderate	2417852	5890552
Cannel Trib 2	Greymouth	6.2	167	5	Moderate	2367180	5870720
Soldiers Creek	Blackball	6.4	177	2	Moderate	2378742	5869947
Cannel Creek Trib. 1	Greymouth	6.5	146	1	Moderate	2366592	5870540
Sullivans Mine Adit	Westport	2.7	963	4	Severe	2408660	5935880

Bathhouse Stream	Westport	2.9	1204	2	Severe	2415720	5951975
Granity Creek	Westport	2.9	814	2	Severe	2414330	5952050
Pack Track Start	Westport	2.9	1220	2	Severe	2416175	5951715
Millers Creek	Westport	3.0	716	2	Severe	2416443	5951573
Garvey	Reefton	3.3	425	5	Severe	2423029	5893567
Mine Creek	Westport	3.3	428	3	Severe	2415395	5952020
Warne Creek	Westport	3.5	486	2	Severe	2422215	5957335
Cannel Creek 2	Greymouth	3.6	559	3	Severe	2366535	5871585
Mine Drainage Causeway	Reefton	3.9	536	5	Severe	2418090	5900730



Appendix 2. A CCA graph showing the relationship between the environmental variables collected and the macroalgal community data set (51 sites).



Appendix 3. A CCA graph showing the relationship between the environmental and community matrices (51 sites; Ten Mile Trib 2 was excluded because it was an outlier). Sites grouped according to pH.